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BIOLOGICAL VARIATIONS OF BACTERIA[1]

I. Induced Variations in the Cultural Characters of B. Coli

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Variations in the biological characteristics of the various members of the Colon-typhoid group have been reported by numerous investigators. These range from mere observations as to peculiarities in the fermentative or other biological tests up to actual mutations as interpreted by De Vries.

The most important observations along the latter line are reported by Massini (1907), who isolated a type of *B. coli* he called "*B. coli-mutabilis.*" In his observations, he found this organism would produce flat colorless colonies on Endo's medium, if transplanted every twenty-four hours. When transplanted at a later period, however, it would produce nodular shaped colonies which became red. The colorless colonies always gave rise to colorless colonies when transplanted not later than twenty-four hours, whereas the red colonies once obtained, never gave rise to any but red colonies irrespective of the time of transfer. The knob like colonies appeared only on lactose media though other carbohydrate media were used. He observed a single reversion from the red to the colorless type, which, however, could not be repeated.

Burk (1908) reports the isolation of a similar mutant and records careful observations which were continued over a period of five months.

Of the more interesting reports in literature on modifications of *B. coli* and allied organisms may be mentioned those of Peck-

[1] Read in part, before the meeting of the Society of American Bacteriologists, held at Philadelphia, December, 1914.

ham, Herter, Penfold, Twort, Manfredi and others. The work of the first of these investigators will be mentioned below, in conjunction with the experiments of the writer.

Herter (1910) has shown that sodium benzoate in weak glucose broth considerably inhibits the fermentative activities of *B. coli*, whereas other biological features are but slightly affected. Such action is entirely prevented by the addition of calcium carbonate. He has also shown that there are no gas producers in food stuffs preserved with sodium benzoate, though 22 of 28 samples contained bacteria of some sort. Penfold (1911) has shown similar action in the case of sodium acetate on *B. coli*, *B. enteritidis* and *B. paratyphi* with diminishing and total disappearance of gas formation in the sugars, though the organisms were still capable of producing gas from the corresponding alcohols. This indicated an inhibition or destruction of the enzyme, invertase, without effect upon the gas producing power. Twort (1907) has shown that *B. typhi*, *B. paratyphi* and *B. dysenteriae* when continuously grown in saccharose media will ultimately ferment saccharose. Manfredi (1889) states that fat-containing media impair the vegetative energy of bacteria.

The observations here reported were undertaken in connection with a series of experiments on the biological variations of bacteria, which the writer intends publishing in sections whenever a sufficient amount of interesting material is accumulated to warrant it. Twenty-one different strains of the various bacilli of the colon-typhoid group were used, in the study but this report is confined only to the *B. coli*, of which seven different strains were experimented on. All of these strains were obtained from the American Museum of Natural History, New York, through the kindness of Prof. C.-E. A. Winslow, and were the stock nos. 19, 44, 45, 46, 52, 57, and 95. The bacteria were subjected to continuous growth at 37.5°C. in 3 per cent glucose, 4 per cent sodium chloride and 1.5 per cent sodium sulphate broth. They were also grown in plain broth and then exposed to the action of phenol in the following manner. The culture was first inoculated into 9 cc. of plain nutrient broth

and incubated for three or four days, at which time 1 cc. of 7.5 per cent of phenol was added to the culture. The phenol exposure was limited to two to three minutes at the beginning of the experiment and then the time was gradually ncreased with each transfer until thirty or more minutes time was attained. The exposed culture was then reinoculated into plain broth by pouring over a small quantity (0.5 to 1 cc.) from it. Continuous growth of the *B. coli* in 0.75 per cent of phenol broth was also tried, beginning with 0.25 per cent, with the same general results. The transplanting in all media was carried out every three or four days over periods varying from one to three months, thus allowing from ten to thirty transfers. The experiments were repeated two or three times to assure constant and uniform findings.

Control cultures were carried on in plain broth throughout the experiment. It might be stated at once that there were very slight variations between the original stocks and these control cultures, no more than would be expected as normal variations. These were seen as slightly increased or decreased amounts of gas or acid formation, differences in time of coagulation, or slight changes in the growth on potato. At no time, however, were the biological characteristics markedly changed nor enzyme production completely inhibited simply by continual passage through broth.

The accompanying tables show the results obtained in some of the more typical series of experiments. These tabulations were all made at seventy-two or ninety-six hours after inoculation and were verified again, especially those on potato and in milk, after a week or ten days growth. The tests for indol were made as described below after seven days growth.

TABLE I

Marked effect of glucose on B. Coli No. 45. Phenol gave a moderate amount of variation particularly in milk and gas production, whereas both sodium chlorid and sulphate showed but slight effect

B. COLI NO. 45	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	++	+++	++	+++	++	+	++	++	++	+++	++	++	++	++	+++	+
Glucose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phenol	+++	++	−	−	++	+++	−	−	++	+++	−	+	+	++	+	++	−	−
Sodium chlorid	++	++	++	+++	+++	+++	++	+	+++	+++	+++	+	+++	++	+++	+++	+++	+
Sodium sulphate	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++

TABLE II

Marked effect of glucose, two different series. The power of producing acid in No. 2 is apparently not effected. Moderate action of phenol and the sodium salts, especially in regard to gas formation

B. COLI NO. 44		POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
		Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control		+++	+++	+++	+++	++	+++	++	++	+++	+++	+++	+++	++	++	++	+++	+++	+++
Glucose	(1)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	(2)	−	−	++	+	+++	−	+++	−	+++	−	+++	−	+++	−	+++	−	+++	−
Phenol	(1)	+++	+++	++	+++	++	−	+++	+	++	++	++	++	++	+	+	+++	++	+
	(2)	+	+++	++	−	+	−					+	−					+	−
Sodium chlorid		+++	+++	+++	+++	+	−	+++	+	+++	+	+++	+	+++	+	+++	+	+++	+
Sodium sulphate		+++	+++	+++	+++	+++	+	+++	+	+++	+	+++	+	++	+	+++	+	+++	+

TABLE III

Marked effect of glucose and phenol, slight effect of the sodium salts

B. coli No. 45	Potato		Milk		Glucose		Lactose		Maltose		Saccharose		Dextrin		Mannite		Fermentation tube	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	+++	+++	++	+++	++	+++	+++	+++	++	++	++	+++	+++	+++	+++	+++
Glucose (1)	++	−	+++	+++	−	−	−	−	++	++	−	−	++	++	++	++	+++	+++
Glucose (2)	+	+	++	+++	++	−	++	−	++	−	++	−	++	−	++	−	++	−
Phenol	++	−	+++	+++	+	−	++	++	++	+++	−	−			−	−	−	+
Sodium chlorid	+	+	++	++	+++	+++	+++	+++	++	+++	+++	+	+++	+++	+++	+++	+++	+++
Sodium sulphate	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++

TABLE IV

No effect of glucose, slight effect of phenol, moderate effect of sodium salts, particularly upon the fermentative qualities

B. coli No. 46	Potato		Milk		Glucose		Lactose		Maltose		Saccharose		Dextrin		Mannite		Fermentation tube	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glucose	+	+	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Phenol (1)	−	−	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Phenol (2)	+	++	++	++	++	+	+++	+++	++	+++	++	++	+++	+++	+++	++	+++	++
Sodium chlorid	+	++	++	++	+++	−	++	−	+	−	+	−	++	−	+++	−	+	−
Sodium sulphate	++	++	++	+++	+++	+++	+++	+++	+	−	+++	+	+	−	+	−	+++	+++

TABLE V

Marked effect of phenol, and slight effect of glucose, none of sodium salts

B. coli no. 19	Potato		Milk		Glucose		Lactose		Maltose		Saccharose		Dextrin		Mannite		Fermentation tube	
	Br. Gr.	Disc.	Coag.	Acid	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glucose	−	−	++	+	++	++	++	+	++	+++	++	+	++	++	++	++	+	−
Phenol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sodium chlorid	+++	+++	+++	+++	+++	+++	+++	+	+++	++	+++	++	+++	+++	+++	++	+++	++
Sodium sulphate	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE VI

Slight effect of sodium salts, none of either glucose or phenol

B. coli no. 52	Potato		Milk		Glucose		Lactose		Maltose		Saccharose		Dextrin		Mannite		Fermentation tube	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	++	+++	++	+++	++	+++	+++	+++	++	+	++	+++	++	+++	+++	++
Glucose	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++
Phenol	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	+++	++	++
Sodium chlorid	−	+	++	+	+++	+++	++	+++	+++	+	++	++	+++	++	−	−	++	++
Sodium sulphate	−	+	++	++	+++	+++	+++	+++	−	−	+++	+++	++	++	++	+	++	++

TABLE VII

Slight effect of all agents, principally the sodium salts

B. coli no. 57	Potato		Milk		Glucose		Lactose		Maltose		Saccharose		Dextrin		Mannite		Fermentation tube	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	++	++	+++	++	+++	+++	+++	+++	+++	++	+	++	+++	++	+++	+++	+++
Glucose	+++	++	++	+++	++	++	+++	++	++	++	++	++	++	+++	++	+++	+++	+++
Phenol	++	+++	+	+	+	+	+++	+++	++	+++	++	++	++	+++	++	++	+++	++
Sodium chlorid	+	+	++	++	+++	+++	+++	+++	++	++	+++	++	++	+++	+	−	++	++
Sodium sulphate	+	+	++	++	+++	+++	+++	+++	−	−	+++	+++	++	+++	++	++	++	++

CHANGES IN MORPHOLOGY

During the course of experimentation several of the strains of *B. coli* showed a change in morphology, the individual organisms becoming two and three times the length of those in the controls, somewhat wider and more vacuolated. This however was not constant, as often on the very next sub-culture they would assume their usual morphological appearances. The only other thing noted under this head was the decrease in motility, which was more marked in the phenol broth than in the other media. Very little importance should be attached to this, however, since some of the strains were hardly motile to begin with, and again, too few observations were made to permit of absolute statements.

GROWTH ON POTATO

Glucose seemed to have a special effect upon the character of growth of *B. coli* on this medium. Five of the seven treated strains, showed at best only a very light yellow color or a slight brownish growth on ordinary potato with practically no discoloration of the medium. Very frequently indeed, the glucose affected organisms would give the typical "invisible" growth seen with the *B. typhi*. Both the original stock and the broth control showed the characteristic colon growth on this medium. This change was noted so many times that an explanation based on differences in the composition of the potato can be excluded. Three of these five strains also showed this change after exposure to phenol. One strain of the *B. coli*, which did not change in this respect with either glucose or phenol, showed this same variation after growing in either sodium chloride or sodium sulphate broth.

ACTION IN MILK

Both phenol and glucose diminished the acid production and inhibited the formation of lab enzyme in three of the seven strains of the *B. coli*, either entirely or for a period of at least two weeks. These results were not seen with the use of the strong saline or sodium sulphate broth.

FERMENTATION OF SUGARS

The results obtained with these substances on *B. coli* with reference to variations in sugar fermentations can be best seen in the accompanying tables. The most striking changes here also were seen in those organisms exposed to glucose and phenol. The former completely inhibited both acid and gas formation in all the sugars tested in three different strains. In two others, glucose varied the amount of acid and gas formation, with an occasional complete inhibition in some of the sugars. Phenol inhibited these fermentations in all of the sugars in only one strain, and in four others, diminished this reaction to the point of inhibition at times only and in different carbohydrates. Sodium chloride and sodium sulphate had less effect than did phenol, giving usually slight variations in amount of acid or gas produced with an occasional inhibition.

Inhibition of all the sugar fermentations in any one experiment was almost always accompanied by inhibition of the usual changes in milk, the characteristic growth on potato, and the formation of indol. In other words, the most typical variations were those in which all acid or ferment production was inhibited.

VARIATIONS IN INDOL PRODUCTION

The production of indol is held by many bacteriologists to be as important a biological characteristic of *B. coli* as its fermentations of the sugars, and is even thought to be of greater importance in its differentiation. This reaction, however, even under normal conditions varies considerably in its quantity and time of appearance with most strains, and at times requires more delicate tests than the usual Salkowski method for its determination.

In the experiments here reported it appears that of the variations induced in *B. coli* that of indol production is the first to take place, often disappearing in the third or fourth culture in glucose broth. This does not hold however when the bacteria are grown in the other media, as evidenced below.

Each strain of *B. coli* was grown in plain broth as control, and in glucose, phenol, sodium chloride and sodium sulphate broth and on potato. Thirty-five sub-cultures were made in all. Indol was tested for after the 10th, 15th, 25th, and 35th transfers. The tests for indol were made by inoculating one loop of culture from the respective medium to which each strain was subjected into standard peptone solution, growing for seven days at 37°C. and then testing by the Salkowski method. All the tests were done at the same time using the same batch of peptone solution throughout the experiment.

The results were uniform for all strains and may be readily interpreted from the following table:

TABLE VIII

NUMBER OF TRANSFER	1	10	15	25	35
Control	+++	+++	+++	+++	+++
Glucose broth	+++	−	−	−	−
Phenol broth	+++	++++	++++	++++	++++
NaCl broth*	+++	++	++	+++	++
Na_2SO_4 broth*	+++	++	++	+++	++
Potato	+++	+++	+++	+++	+++

* Exposure to these substances gave variable results, at times an increase and at others a decrease in indol production.

All the controls, grown in plain broth, gave good indol tests even after the 35th sub-culture. Those grown in glucose broth gave none at the 10th sub-culture or thereafter. In phenol broth the property of indol production seemed to be somewhat increased, judging from the intensity of the reaction. Sodium chloride and sodium sulphate, and prolonged cultivation on potato practically exerted no influence, or if any, showed a slight inhibitory effect.

Experiments were then carried out to see how soon the property of indol production is interfered with by growth in 3 per cent glucose broth, and it was found that *B. coli* lost this property usually on the third and at times on the second transfer over a period of from seven to ten days. In one experiment sub-cultures were made every twenty-four hours with a total

disappearance of the indol tests in from forty-eight to seventy-two hours in all the strains.

The tests in these latter experiments were made in the culture tubes themselves, not transferring to the peptone solution, after seven days of growth. In order to exclude the possibility of interference with the indol test by the presence of the glucose, several cultures in both plain broth, and peptone, were made, and grown at 37°C. for seven days. Glucose was added to each of the cultures and they were then tested for indol. Positive tests were obtained in all cases, excluding any possibility of such interference by the presence of the carbohydrate. An interesting observation may also be mentioned at this juncture. Cultures of the organisms in plain broth of seven days growth to which phenol or sodium chloride were added showed a decided increase in the indol reaction in case of the phenol and a diminished reaction in the tubes to which the sodium chloride was added. In the interpretation of these tests comparison was made with controls. It may be possible that the presence of these substances intensifies or diminishes the color produced, the differences not being due to actual variations in the amount of indol formed. The different culture media themselves were tested for indol, after incubating for seven days, for the purpose of control and they were found negative.

Experiments were then carried out to determine the permanency of this change. The cultures in glucose broth after the 35th transfer were grown in plain broth, transplanting every day and tested on the seventh day of incubation. Four of the strains of *B. coli*; nos. 44, 45, 46 and 52 gave slight indol reactions on the third transfer, no. 46 gave a good positive on the fifth transfer, but the others took from five to ten more transfers before they could be called "+" or "++" positive. Nos. 57 and 95 took six transfers before a trace of indol appeared. No. 19, a very feeble indol producer in the control, remained negative up to the fifteenth transfer at which time the experiment was discontinued.

Investigations as to the agglutinability of these altered strains of *B. coli* were also made, but the work is too meagre and the

results too indefinite to be reported at the present time. The writer intends to continue work along this line, and also with respect to pathogenicity, which seems to suggest itself as a fruitful subject for investigation.

In summing up, it can be said that glucose and phenol, particularly the former, cause partial inhibition or total disappearance of acid and enzyme formation in some strains of *B. coli*. These changes together with the suspension of the production of indol and the characteristic colon growth on potato, makes the *B. coli* approach the *B. typhi* type. These changes have been noted time and again but in varying degrees, in those strains

TABLE IX

Changes produced by glucose and phenol in various strains of B. coli and the complete or incomplete reversion towards their previous biological characteristics. The completeness of the change and the incompleteness of the reversion in strain No. 95 is noteworthy, as such a change might be regarded in the light of a mutation instead of a variation

	POTATO		MILK		GLUCOSE		SACCHAROSE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas
B. Coli, No. 95										
Control.....	+++	+++	+++	+++	++	+++	++	++	+++	+++
Glucose.....	−	−	++	++	++	−	++	−	++	−
Reversion....	−	−	++	++	−	−	+	−	−	−
B. Coli. No. 19										
Control.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glucose.....	−	−	−	−	−	−	−	−	−	−
Reversion....	−	−	+++	+++	+++	++	++	+++	+++	++
B. Coli. No. 44										
Control.....	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
Glucose.....	−	−	−	−	−	−	−	−	−	−
Reversion....	+++	+	++	+++	+++	+++	++	Sl.*	+	Sl.
Phenol.....	+++	+++	++	+++	+	−	++	−	−	+
Reversion....	++	++	+++	+++	++	+	++	++	+	+
B. Coli. No. 45										
Control.....	+++	+++	++	+++	++	+++	++	+++	+++	+
Glucose.....	−	−	−	−	−	−	−	−	−	−
Reversion....	−	−	++	Sl.	+	+	+	+	+	+
Phenol.....	+++	++	−	−	++	+++	−	−	−	−
Reversion....	++	++	++	Sl.	+	+	+	+	+++	+

* Sl. = slight.

that are susceptible to variations, but for some unexplained reason cannot be regarded as altogether constant. Indol formation would invariably return when these altered bacteria were transplanted into plain broth at frequent intervals. Lab enzyme would also return in most of the altered strains but not invariably so. The same can be said of the fermentative properties. Very often, however, these characteristics appear to be entirely done away with, the change being permanent as far as could be made evident by sub-culturing into plain broth. (See Table IX.) In these cases observations were made up to two months after the last exposure to the influencing substance, making frequent transfers. There seemed to be no definite rule of reversion, and no relation between the reappearance of one enzyme and another. The reappearance of the fermenting enzymes in one sugar was not necessarily accompanied by those in other sugars. At times the fermentation of one sugar might have returned to nearly normal, while others might show little or no presence of gas with the same strain of *B. coli*.

ADDENDA

The work under this heading was undertaken as supplementary to the foregoing section for the purpose of verification and also in response to comments made upon the report of this paper at the meeting of the Society of American Bacteriologists, held at Philadelphia, in December, 1914.

To avoid any objection arising as to the possible existence of mixed cultures, or of "weak members" at the start, each strain of *B. coli* was plated, a single colony selected, and replated, and from this latter plate, several cultures were selected, inoculated on agar slants and after twenty-four hours cultivation, carried through on all media. The strain that showed the greatest amount of enzyme formation was selected as the "strongest" and the one to be subjected to experimentation.

In general, the technique of these experiments was identical with that already described, observations being made with special reference to indol production which was taken as the

index of proteolytic activities. To control the possible effect of the acid production of *B. coli*, upon proteolysis, three of the sugars, namely, glucose, lactose, and saccharose, had 1 per cent of calcium carbonate added to neutralize any acid formed. Another control was also carried along, a stock strain of *Sp. cholerae*, which produces but a slight amount of acid as compared with *B. coli*, but, on the other hand, gives a marked amount of indol. Seven different sugars were used besides the three containing calcium carbonate, making a total of ten inoculations for each organism. The organisms were cultivated in the respective sugar peptone solutions for six days, at which time small amounts of the cultures were poured over into fresh media for the continuance of the experiment, and then the Salkowski's test was applied to the original culture. After the seventh inoculation, when all the strains gave negative indol tests, experiments for reversions were begun, by transfering a small quantity of each of the cultures into plain peptone, and proceeding with the test as before.

TABLE X

Showing the effect of various carbohydrates upon the proteolytic activities of seven different strains of B. coli, and one of Sp. cholerae, as evidenced by the indol test

SUBCULTURE	CONTROL			GLUCOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1.....	+	tr.	+	–	–	–	–	–	–	–	–	–	–
B. coli No. 2.....	++	++	++	tr.	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 3.....	+++	++	+++	–	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 4.....	++	++	++	tr.	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 5.....	+++	+++	+++	tr.	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 6.....	+++	+++	++	–	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 7.....	++	++	++	tr.	–	–	tr.	–	–	–	–	–	–
Sp. cholerae.....	+++++	++++	+++++	tr.	–	–	tr.	–	–	–	–	–	–

TABLE XI.

*Results of the indol tests in the experiments on reversion. * = Positive test obtained with H_2SO_4 only. † = Positive test obtained only after the addition of nitrite*

B. coli No. 1.....				–	–	–	–	–	–	–	tr.	–	+
B. coli No. 2.....				–	–	?	?	–	sl. tr.	sl. tr.	–	–	–
B. coli No. 3.....				–	–	?	–	?	–	–	?	–	–
B. coli No. 4.....				–	–	–	–	?	–	–	–	–	?
B. coli No. 5.....				–	–	–	–	?	–	–	?	tr.	+
B. coli No. 6.....				–	–	–	–	?	–	–	–	?	?
B. coli No. 7.....				–	–	sl. tr.	sl. tr.	–	–	–	sl. tr.	–	–
Sp. cholerae.....				–	–	?	–	sl. tr.	tr.	tr.†	+	+	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			GLUCOSE + $CaCO_3$									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1	+	tr.	+	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 2	++	++	++	tr.	?	?	tr.	–	–	–	–	–	–
B. coli No. 3	+++	++	+++	tr.	tr.	?	tr.	–	–	–	–	–	–
B. coli No. 4	++	++	++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 5	+++	+++	+++	tr.	tr.	?	tr.	–	–	–	–	–	–
B. coli No. 6	+++	+++	++	tr.	?	?	tr.	–	–	–	–	–	–
B. coli No. 7	++	++	++	tr.	tr.	?	tr.	–	–	–	–	–	–
B. cholerae	+++++	++++	+++++	+	tr.	–	–	–	–	–	–	–	–

TABLE XI—*Continued*
Reversion from above

B. coli No. 1				–	–	sl. tr.	–	tr.	–	tr.*	tr.*	–	tr.*
B. coli No. 2				–	–	–	–	tr.	–	–	–	–	–
B. coli No. 3				–	–	sl. tr.	++	++	–	–	–	–	–
B. coli No. 4				–	–	tr.	+++	++	–	–	–	–	–
B. coli No. 5				–	–	?	++	++	–	–	–		
B. coli No. 6				–	–	?	++	++					
B. coli No. 7				–	–	tr.	++	++					
B. cholerae				–	–	–	–	sl. tr.	–	tr.*	tr.*	tr.*	tr.*

TABLE X—*Continued*

SUBCULTURE	CONTROL			LACTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1	+	tr.	+	–	–	–	–	–	–	–	–	–	–
B. coli No. 2	++	++	++	tr.	tr.	?	tr.	–	–	–	–	–	–
B. coli No. 3	+++	++	+++	+	tr.	tr.	tr.	–	–	–	–	–	–
B. coli No. 4	++	++	++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 5	+++	+++	+++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 6	+++	+++	++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 7	++	++	++	tr.	–	?	tr.	–	–	–	–	–	–
B. cholerae	+++++	++++	+++++	++	–	tr.	tr.	–	–	–	–	–	–

TABLE XI—*Continued*
Reversion from above

B. coli No. 1				–	–	–	tr.	sl. tr.	tr.	+*	+*	++	tr.
B. coli No. 2				–	–	tr.	+	sl. tr.	tr.	+*	tr.*	+	+
B. coli No. 3				–	–	sl. tr.	+	+	–	+			
B. coli No. 4				–	–	sl. tr.	+	++	+	+			
B. coli No. 5				–	–	?	+	++	+	++			
B. coli No. 6				–	–	?	+	+	+	+			
B. coli No. 7				–	–	sl. tr.	+	+	+	+			
Sp. cholerae				–	–	sl. tr.	+	+	+	+†	tr.†	+	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			LACTOSE + $CaCO_3$									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1.....	+	tr.	+	–	–	–	–	–	–	–	–	–	–
B. coli No. 2.....	++	++	++	tr.	tr.	?	tr.	–	–	–	–	–	–
B. coli No. 3.....	+++	++	+++	tr.	–	?	tr.	–	–	–	–	–	–
B. coli No. 4.....	++	++	++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 5.....	+++	+++	+++	tr.	?	–	tr.	–	–	–	–	–	–
B. coli No. 6.....	+++	+++	++	tr.	?	?	tr.	–	–	–	–	–	–
B. coli No. 7.....	++	++	++	tr.	tr.	–	–	–	–	–	–	–	–
Sp. cholerae.....	+++++	++++	+++++	+	tr.	+	tr.	tr.	–	–	–	–	–

TABLE XI—*Continued*

Reversion from above

B. coli No. 1.....				–	–	?	–	–	–	–	–	–	–
B. coli No. 2.....				–	–	sl. tr.	sl. tr.	sl. tr.	tr.	+*	+*	+	+
B. coli No. 3.....				–	–	tr.	+	++	+				
B. coli No. 4.....				–	–	?	+	++	+				
B. coli No. 5.....				–	–	sl. tr.	+	+	+				
B. coli No. 6.....				–	–	–	+	+	+				
B. coli No. 7.....				–	–	sl. tr.	+	+	+				
Sp. cholerae.....				–	–	sl. tr.	tr.	+	+	tr.	sl. tr.	tr.	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			SACCHAROSE																
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
B. coli No. 1.	+	tr.	+	tr.	–	tr.	–	–	–	–	–	–	–								
B. coli No. 2.	++	++	++	tr.	tr.	–	tr.	–	–	–	–	–	–								
B. coli No. 3.	+++	++	+++	++	+	+	+	+	+	tr.	+	+	+	+	+	tr.	+	+	tr.	sl. tr.	sl. tr.
B. coli No. 4.	++	++	++	tr.	tr.	–	tr.	–	–	–	–	–	–								
B. coli No. 5.	+++	+++	+++	++	+	+	+	+	+	tr.	+	+	+	+	+	+	+	tr.	+	tr.	+
B. coli No. 6.	+++	+++	++	++	+	+	+	+	+	+	+	+	+	tr.	+	+	+	tr.	tr.	+	tr.
B. coli No. 7.	++	++	++	tr.	–	–	tr.	–	–	–	–	–	–								
Sp. cholerae..	+++++	++++	+++++	+	tr.	–	tr.	–	–	–	–	–	–								

TABLE XI—*Continued*

Reversion from above

B. coli No. 1...				–	–	–	–	–	–	–	–	tr.	sl. tr.							
B. coli No. 2...				–	–	–	–	tr.	+*	tr*	+*	tr.	tr.							
B. coli No. 3...				*																
B. coli No. 4...				–	–	+	++	++	+											
B. coli No. 5...				*																
B. coli No. 6...				*																
B. coli No. 7...				–	–	+	++	+	+											
Sp. cholerae...				–	–	+†	tr.	sl. tr.	sl. tr.	tr.†	tr.†	tr.	tr.							

TABLE X—*Continued*

SUBCULTURE	CONTROL			SACCHAROSE + $CaCO_3$											
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12
B. coli No. 1....	+	tr.	+	tr.	–	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 2....	++	++	++	–	tr.	–	tr.	–	–	–	–	–	–	–	–
B. coli No. 3....	+++	++	+++	+	+	+	+	+	+	tr.	+	+	tr.	+	tr.
B. coli No. 4....	++	++	++	tr.	tr.	?	tr.	+	–	–	–	–	–	–	–
B. coli No. 5....	+++	+++	+++	+	+	?	+	+	+	+	tr.	+	tr.	tr.	tr.
B. coli No. 6....	+++	+++	++	+	+	+	+	+	+	+	+	tr.	+	+	+
B. coli No. 7....	++	++	++	tr.	tr.	?	tr.	–	–	–	–	–	–	–	–
Sp. cholerae....	+++++	++++	+++++	+	tr.	tr.	tr.	tr.	–	–	–	–	–	–	–

TABLE XI—*Continued*

Reversion from above

B. coli No. 1....				–	–	–	–	–	–	–	–	tr.*	–	tr.*	–
B. coli No. 2....				–	–	sl.tr.	tr.	tr.	tr.*	tr.	+*	+*	+*	+*	+*
B. coli No. 3....				*											
B. coli No. 4....				+	+	+	+	+	+	+	+	tr.	+	+	*
B. coli No. 5. ..				–	–	–	?	tr.	tr.	+	+	tr.	+		
B. coli No. 6....				*											
B. coli No. 7....				–	–	tr.	+++	++	+	+					
Sp. cholerae....				–	–	–	sl. tr.	tr.	tr.	tr.†	tr.	+	tr.	+	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			MALTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1.....	+	tr.	+	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 2.....	++	++	++	tr.	tr.	?	?	?	–	–	–	–	–
B. coli No. 3.....	+++	++	+++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 4.....	++	++	++	–	tr.	?	?	?	–	–	–	–	–
B. coli No. 5.....	+++	+++	+++	tr.	tr.	?	?	–	–	–	–	–	–
B. coli No. 6.....	+++	+++	++	tr.	tr.	?	?	–	–	–	–	–	–
B. coli No. 7.....	++	++	++	tr.	tr.	?	?	–	–	–	–	–	–
Sp. cholerae.....	+++++	++++	+++++	+	tr.	?	?	–	–	–	–	–	–

TABLE XI—*Continued*

Reversion from above

B. coli No. 1.....				–	–	sl. tr.	–	–	sl. tr.	sl. tr*	–	tr.	tr.
B. coli No. 2.....				–	–	sl. tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
B. coli No. 3.....				tr.	–	tr.	+	++	+				
B. coli No. 4.....				–	–	+	+	+	+				
B. coli No. 5.....				–	–	+	++	+	+				
B. coli No. 6.....				–	–	+	++	+	+				
B. coli No. 7.....				–	tr.	+	++	+	+				
Sp. cholerae.....				–	–	–	tr.	tr.	tr.	tr.†	tr.†	tr.	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			GALACTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1	+	tr.	+	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 2	++	++	++	–	tr.	–	–	–	–	–	–	–	–
B. coli No. 3	+++	++	+++	tr.	?	–	–	–	–	–	–	–	–
B. coli No. 4	++	++	++	tr.	?	–	–	–	–	–	–	–	–
B. coli No. 5	+++	+++	+++	tr.	?	–	–	–	–	–	–	–	–
B. coli No. 6	+++	+++	++	tr.	?	–	–	–	–	–	–	–	–
B. coli No. 7	++	++	++	tr.	?	–	–	–	–	–	–	–	–
Sp. cholerae	+++++	++++	+++++	–	?	–	–	–	–	–	–	–	–

TABLE XI—*Continued*

Reversion from above

B. coli No. 1				–	–	sl. tr.	+	+	+	tr.	tr.	++	+
B. coli No. 2				–	–	–	–	–	tr.	tr.	–	tr.	sl. tr.
B. coli No. 3				–	–	sl. tr	+	+	+				
B. coli No. 4				–	–	sl. tr.	++	+	+				
B. coli No. 5				–	–	?	+	+	+				
B. coli No. 6				–	–	+	+	+	+				
B. coli No. 7				–	–	tr.	+	+	+				
Sp. cholerae				–	–	–	–	–	+	tr.	tr.	tr.	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			DEXTRIN									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1	+	tr.	+	–	–	–	–	–	–	–	–	–	–
B. coli No. 2	++	++	++	tr.	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 3	+++	++	+++	tr.	tr.	–	tr.	–	–	–	–	–	–
B. coli No. 4	++	++	++	tr.	tr.	–	–	–	–	–	–	–	–
B. coli No. 5	+++	+++	+++	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 6	+++	+++	++	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 7	++	++	++	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	?
Sp. cholerae	+++++	++++	+++++	tr.	–	–	tr.	–	–	–	–	–	–

TABLE XI—*Continued*

Reversion from above

B. coli No. 1				–	–	–	–	+°	++°	+°	+°	++°	+
B. coli No. 2				–	–	tr.	–	sl. tr.	tr.	+°	tr.°	+	+
B. coli No. 3				+	+	+	++	+	+				
B. coli No. 4				+	+	+	+	+	+				
B. coli No. 5				+	+	+	++	+	+				
B. coli No. 6				+	+	+	++	++	+				
B. coli No. 7				+	+	+	++	+	+				
Sp. cholerae				–	–	sl. tr.	sl. tr.	sl. tr.	tr.	tr.	tr.†	+	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			MANNITE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1....	+	tr.	+	tr.	–	–	–	–	–	–	–	–	–
B. coli No. 2....	++	++	++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 3....	+++	++	+++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 4....	++	++	++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 5....	+++	+++	+++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 6....	+++	+++	++	tr.	tr.	?	–	–	–	–	–	–	–
B. coli No. 7....	++	++	++	tr.	tr.	?	–	–	–	–	–	–	–
Sp. cholerae....	+++++	++++	+++++	+	tr.	?	–	–	–	–	–	–	–

TABLE XI—*Continued*
Reversion from above

B. coli No. 1....				–	–	–	–	–	–	–	tr.	–	–
B. coli No. 2....				–	–	+	+	+	+	tr.*	++	+	+
B. coli No. 3....				–	–	+	+	+	+				
B. coli No. 4....				–	–	+	+	+	+				
B. coli No. 5....				–	–	sl. tr.	+	+	+				
B. coli No. 6....				–	–	+	+	+	+				
B. coli No. 7....				–	–	tr.	+	+	+				
Sp. cholerae....				††	tr.†	+++†	++†	+++†	++†	+	++†	++†	++†

A study of the accompanying tables will best convey to the reader the results obtained. It will be noted that, strain no. 1 is the "weakest" member of the group, giving but little indol in the controls and but traces in five of the ten sugar media used. Strains 2, 4 and 7 are readily changed by all sugars, with a rapid disappearance of indol formation. The most interesting results, however, were seen in strains 3, 5 and 6. These were affected similarly to nos. 2, 4 and 7 by all the sugars except saccharose, in which medium the indol reaction continued in each case until the 18th transfer, at which time the experiment was discontinued. It was suspected that these latter were strains of *B. coli communis*, and since *B. communis* normally does not ferment saccharose with the production of gas, possibly not affected by this carbohydrate. Inoculating the original agar culture of nos. 3, 5 and 6 into saccharose media proved this supposition to be correct. At the time of the seventh transfer, the amount of indol produced by these three strains was markedly less than at the beginning of the experiment. This, coupled with the fact that the bacteria were

subjected to saccharose for over forty days, suggested the possibility that they had acquired the function of fermenting saccharose, and that the resultant glucose, in its turn, had its effect upon the indol production as evidenced in the other experiments. Planting these organisms after the seventh subculture into saccharose agar proved this to be the case, as each of the strains, 3, 5 and 6, now fermented saccharose with the production of gas to a moderate degree.

The writer desires to emphasize at this juncture what appears to him a certainty, namely, that it is the carbohydrate per se that causes the fluctuating biological modification noted in those experiments, though a definite *modus operandi* is not known. This is well seen in the action of saccharose which had but slight effect upon three of seven strains of *B. coli* until the organisms were capable of splitting it into glucose, which, in its turn, effected the amount of indol produced. The fact that the inhibition was not complete, does not contradict this supposition, for it can be accounted for either by the presence of individual bacteria that remained unaltered by virtue of their greater resistance, or by the fact that the attack on saccharose was sufficiently slow to permit a small amount of proteolytic cleavage. Further proof of such effects of carbohydrates will be offered below.

The cholera spirillum, though it produced a large amount of indol in the control, readily succumbed to the action of the various carbohydrates, and, in several instances, even sooner than the *B. coli*. The fact that this strain of cholera spirillum produced but very little acid would indicate that the inhibitory action was not due to acid accumulation. The possible effect of excess acid is likewise shown to be negligible by the results obtained in the carbohydrate media, wherein, as may be seen by consulting the tables, the differences were so slight that they cannot be regarded as having any bearing.

It is of interest to note the differences in the action of the various carbohydrates. Saccharose has already been commented upon. Galactose, it appears, had the most pronounced effect upon all of the organisms, permitting but a single trace of indol

in five of the eight organisms in the first culture tube, and practically none thereafter. Dextrin, on the other hand, showed the greatest variation in its effect upon the different strains of *B. coli*. Strain no. 1 gave no indol throughout the experiment; both nos. 5 and 6 gave a single trace in the first culture but none thereafter, whereas no. 7 gave a trace in each sub-culture up to the tenth, at which time the experiment was discontinued. Maltose and mannite appear to have a more pronounced inhibitory effect upon indol formation than either glucose, lactose or saccharose and would stand intermediate between them and galactose.

Experiments were undertaken to determine the permanency of this change in biological activities using the same technique as previously described. The bacteria, after being carried through the carbohydrate peptone media for at least seven transfers, were then inoculated into plain peptone, making successive transfers until three consecutive positive indol tests were obtained.

By referring to Table XI, the most striking difference is seen between the action of glucose and dextrin. In the case of glucose the indol reaction reappeared only in the cases of *B. coli* nos. 1, 2, 5 and 7, and the cholera spirillum, the others remaining negative up to the tenth transfer in plain peptone, at which time the experiment was discontinued. For some unexplained reason, the re-appearance of the property of indol formation was slower and less marked in this set of experiments than on previous occasions. When contrasted with the action of dextrin, a marked difference is seen. This substance permits a prompt return of the indol producing property in the first transfer of the different strains of bacteria used, with the exception of *B. coli* nos. 1 and 2, and the cholera spirillum. Inasmuch as this peculiarity was evident in each of the sugars, it would appear that it was not due to the carbohydrate, but to a possible greater susceptibility on the part of these strains of bacteria. *B. coli* nos. 3, 4, 5, 6 and 7 gave positive indol tests on the third or fourth transfers quite uniformly in all sugars except glucose as already mentioned. It may be concluded that dextrin acts entirely unlike the other carbohydrates, having, in

the first place, a somewhat selective action on the different strains of *B. coli*, and secondly, permitting an immediate return to normal proteolytic activity, under the conditions of experimentation here outlined.

Aside from the peculiar manner in which *B. coli* nos. 1 and 2 and the cholera spirillum acted on reversion in contrast to the other strains, there is another observation worthy of mention. It was noted that frequently in doing the Salkowski test with these colon strains, a positive reaction occurred without the addition of nitrite. This is rarely found with the *B. coli* and had not been noticed in any of the control cultures nor in the previous experiments. This peculiarity of the test was quite irregular in its occurrence, it appeared as if at will, and disappeared and re-appeared without any apparent reason, since the conditions of the experiment were unaltered. The reverse was often noted with the cholera spirillum, in which case, instead of obtaining a positive indol test on the addition of acid alone, none occurred until nitrite was added. This observation would indicate some changes in the power of nitrite formation entirely independent of that of indol production, and would well fit in with the carbohydrate effect upon the other biological activities already mentioned.

In order further to ascertain the action of the various sugars on the biological activities of the different strains of bacteria in question, a proteid-free medium was used as a base, to which 3 per cent of the different sugars was added. Those used were glucose, lactose, saccharose, maltose, and dextrin. Inoculations were made from the same stock cultures of *B. coli* as in the above experiments, a control of each strain being carried in the proteid-free medium itself. The medium selected was the writer's modification of Naegeli's proteid-free medium and had the following composition:

Ammonium tartrate	10.0
Potassium phosphate	1.0
Magnesium sulphate	0.2
Calcium chloride	0.12
Glycerin	20.0
Water	1000.0

The medium base was made up in moderate quantities, sterilized in the autoclave and kept until needed. The sugars were added to separate small quantities as required, tubed and sterilized by the intermittent method. Inoculations were made every fourth or fifth day over a period of fifteen weeks, making a total of twenty-six transfers. Tests were made from the last culture tube to determine biological variations by planting into the various laboratory media, final observations being noted after seven days inoculation.

This series of experiments was not as successful as the writer desired. Many of the strains suddenly refused to grow, and were lost at different stages of the experiment. This can be explained as due either to a lack of proper nutrition, or, possibly, to an inhibitory action of the sugars, or both. "Sudden death" was also noted by Peckham (1897) in her experiments with *B. coli* grown in glucose peptone, and is accounted for by her as due to the accumulation of by-products coupled with the complete exhaustion of the bacteria. She believes that on account of incomplete proteolytic activities, through the preference for the carbohydrate food, there is a deficiency in plastic material of the bacteria sufficient to interfere with reproduction and building up of the cells. Besides the loss of a number of strains, several were found to be contaminated and had to be discarded.

Though but five cultures were subjected to the carbohydrate action, and two controls were carried through to the completion of the experiment, the writer believes the results worthy of record, particularly since they verify the results obtained in the work above reported. This is also desirable since the intent of these experiments was to show the ability of the controls to retain their various biological characteristics even though subjected to carbohydrates.[2]

Table XII indicates the results obtained. Strains 3 and 6, subjected to the action of glucose in a proteid-free medium, seem to compare well with the results obtained with glucose

[2] These experiments are being repeated, and will again be reported on at a later date.

TABLE XII

Action of carbohydrates in a peptone free medium upon the cultural characteristics of several strains of B. coli-marked inhibition by glucose, lactose and maltose, in strain No. 3, and only slight by glucose and saccharose in strain No. 6

	STRAIN NO.	POTATO		MILK		GLUCOSE		LACTOSE		SACCHAROSE		MALTOSE		DEXTRIN		MANNITE		FERMENTATION TUBE		INDOL
		Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	
Control.....	3	++	+++	+++	+++	++	++	++	++	++	−	+++	++	+++	++	+++	++	++	+++	+
Glucose.....	3	−	−	+	−	++	−	−	−	+	−	++	−	−	−	++	−	+	−	−
Glucose.....	6	++	++	+++	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	−
Lactose.....	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Saccharose .	5	++	++	++	++	+++	++	+++	++	+	+	++	+	++	+	+++	+++	−	−	−
Control.....	1	+++	++	+++	+++	+++	++	+++	+++	++	−	+++	++	++	++	++	++	+++	+++	S1
Maltose.....	1	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−

broth media. *B. coli* no. 3 is markedly changed from its control, which itself appears weaker in its cultural characteristics than controls carried in broth. The changes in the other strains need but little comment. When compared with the glucose action as seen in the previous tables, a similar action may be noted here.

Too much stress cannot be placed upon so few experiments with proteid-free media, but the writer feels confident that inhibitory action of other sugars than glucose does occur, and can readily be demonstrated. It may be well in passing, to note that indol production is invariably completely suppressed. Next to indol, the greatest amount of inhibition is manifest upon gas production, then, on lab enzyme and the characteristic growth on potato and finally on acid production. This sequence is not always adhered to, but holds good in a general way. All of these activities seem to correspond very well indeed with the action of glucose in broth media.

Peckham in a series of experiments along similar lines prefers to regard the changes as not due to any direct carbohydrate, or chemical effect, but rather to what she terms exhaustion, sheer inability to produce normal biological effects on account of previously expended energy. She describes numerous experiments carried out with a number of strains of *B. coli*, in which she claims that proteolytic activities were suspended when these organisms were grown in fresh peptone sugar broth. The index she took for determining proteolysis was the amount of indol produced by those subjected to carbohydrate as compared to control cultures. She concluded that this suppression of proteolytic activities was due to the preference of the *B. coli* for carbohydrate, a more readily assimilated food, to proteins, with a subsequent exhaustion on the part of the bacteria by the time they reached the protein material. That this "exhaustion of energy" was not due to the amount of lactic acid produced was proven by her in another series of tests in which quantities of lactic acid were added to similar peptone solutions without carbohydrate, and in which indol was produced as in the control cultures.

This preference for carbohydrate food to the exclusion of proteolysis has also been shown by other investigators to occur both with *B. coli* and numerous other bacteria, but in the opinion of the writer there is still an open question in regard to the *modus operandi*, namely, whether it is due to sheer exhaustion, or to a certain yet unexplained carbohydrate effect upon the metabolic activities of the bacteria.

Peckham did not return the *B. coli* into plain peptone to determine whether the absence of indol was due to a simple suspension or inhibition of proteolytic activities, or whether this inhibition was a pronounced one extending over a period of time. Had she done so, she would probably have found, as did the writer, that this inhibition was a decided one, not readily overcome, that it varied with different strains of *B. coli*, and also varied with the carbohydrate used. In the light of the experiments here reported the writer feels quite convinced that exhaustion, though it may be given a certain amount of credit, cannot explain all of his findings. This contention is emphasized by: (1) The different effects obtained on different strains of *B. coli*. (2) Differences in effect by the various sugars, dextrin acting much more readily, and permitting an immediate return of indol formation, whereas in the case of glucose, the carbohydrate effect was more gradual and more lasting. (3) The fact that in an occasional experiment, the organism would not revert to its original type, but remain permanently changed in some or even all of its biological characteristics. (4) Differences in the quantity, and time of disappearance or reappearance of the various enzyme activities, with no special sequence of events. (5) Exhaustion, though in spite of the above factors it might still be regarded as being the cause in regard to indol, cannot explain the more gradual loss of lab enzyme, fermentation of carbohydrates with the production of gas, the lack of typical growth on potato and finally loss of acid production. If we were still to insist that it is a matter of exhaustion, we must assume that it was so profound that it could have been handed down from one culture to another over certain periods of time as evidenced in the experiments for reversion. This would

be contrary to any existing ideas in respect to the effects of exhaustion and would intimate that exhaustion can be inherited.

The following conclusions may be drawn from the foregoing experiments with *B. coli*.

1. Both glucose and phenol give rise to either partial or complete inhibition of the cultural characteristics of some strains of *B. coli*. Sodium chloride and sodium sulphate also display inhibitory action but by no means as marked as either of the above compounds.

2. When complete, the change is more lasting, but there is always present a strong tendency for the modified bacteria to return to their former status of biological activities.

3. Occasionally, complete reversion does not take place, in which case the organism remains permanently devoid of certain enzymes or of the power of fermenting one or more of the carbohydrates.

4. There is no well defined nor constant sequence of events either during the process of modification or reversion, and no relationship between the changes produced in the various enzymes, but, it may be stated, in a general way, that indol production is the first to disappear, then, the fermentations of the various carbohydrates, the characteristic growth on potato, lab enzyme, and finally, acid production.

In closing, the writer desires to take this opportunity of expressing his sincerest thanks to his students, Messrs. Bingaman, Braude, Denehey, Nachamofsky, Rubinsky, Russo, and Miss Wright, for their kind coöperation in the above work.

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A NEW CULTURE MEDIUM FOR THE TUBERCLE BACILLUS

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Contamination is an occurrence which has always troubled bacteriologists in the isolation and cultivation of the tubercle bacillus. Even the various digestive methods which are used at present, such as the antiformin, the sodium carbonate, and the sodium hydroxid methods, have failed to yield uniformly satisfactory results. It is obvious that the ideal culture medium for this purpose would be one which included some substance that had the property of preventing the growth of other microorganisms while not interfering with the isolation and reproduction of the tubercle bacillus.

The observation of v. Drigalski and Conradi (1902), that crystal violet was able to inhibit the growth of many bacteria but had no effect on the cultivation of typhoid and colon bacilli was the first step in this direction. Then followed the splendid researches of Churchman (1912) who worked with a number of common dyes and discovered that gentian violet possessed a striking selective power. He found that by making divided plates, one-half filled with plain agar and the other filled with agar to which 0.001 per cent gentian violet was added, and stroking the surface with a mixed culture, he could get rid of the contaminating organisms. For instance, by this means he purified a culture of *B. tuberculosis* which had become contaminated with *B. subtilis*. The former organism is "gentian-negative" while the latter, like almost all air contaminations, is "gentian-positive." He states that the effect of gentian violet on gentian-positive organisms is better described as bacteriostatic, rather than bactericidal, meaning that the dye suspends repro-

duction without implying that the organisms are necessarily killed. He finds that this gentian reaction is much more definite and constant than the Gram stain.

Next came the attempt of Petroff (1915) to devise a simple, practical and reliable method for the isolation and cultivation of the tubercle bacillus from the sputum and feces. He experimented with gentian violet, methyl violet, methylene blue, crystal violet, and fuchsin added to a meat-juice-glycerin -egg medium. He found that gentian violet was the most favorable stain on account of its inhibitory action on many organisms and reported that he obtained sixty-nine positive cultures from sixty-nine specimens of sputum from practically all stages of tuberculosis. Six of these specimens were negative by direct microscopic examination.

Shortly after the publication of Petroff's article, we began the use of his method. We were soon impressed with the inhibitory effect of the sodium hydroxid and gentian violet which he used, but not with the medium as a whole. It did not seem to contain enough moisture to prevent rapid drying; it required inspissation on three successive days, with the result that frequently an uneven surface was obtained in spite of the utmost care; also, it occasionally became contaminated with spore bearing organisms which would usually cause liquefaction of the medium with consequent destruction of hopeful cultures.

It seemed to us that if a gentian violet medium could be made which did not require inspissation, gave a smooth surface, contained sufficient moisture, and could be sterilized in an autoclave, we should have a nearly ideal culture medium.

While working with the medium devised by Besredka (1913) for growing tubercle bacilli to make his tuberculin, which serves as a good antigen in tuberculosis complement fixation tests, it occurred to us to utilize it in an attempt to overcome the defects of Petroff's medium. We believe we have accomplished this by making a medium as here described.

PREPARATION OF THE MEDIUM

1. Egg-white solution. This is made by diluting the egg-white with ten parts of distilled water and thoroughly shaking. The fluid is opalescent and contains numerous whitish flakes. To clear it, it is passed through a thin layer of cotton and then heated to 100°C. to hasten precipitation. It is then filtered through paper.

2. Egg-yolk solution. The yolks are diluted with ten parts of water and well stirred. The very cloudy emulsion is clarified by adding normal sodium hydroxid. Too much hydroxid is harmful and therefore complete solution of the yolk is not desirable. The emulsion should be slightly turbid. To attain the proper degree of turbidity, one cubic centimeter of normal sodium hydroxid is usually added to each one hundred cubic centimeters of the emulsion. This is not a constant amount, however, because some yolks will be completely dissolved by less than half this amount of alkali. The solution is heated to 100°C. and filtered.

3. Meat infusion. Five hundred grams of finely chopped lean veal are covered with one liter of water containing 15 per cent of glycerin, allowed to infuse for twenty-four hours and filtered; 5 grams of sodium chlorid are added, and the infusion heated to boiling. It is again filtered and then rendered plus 1 per cent alkaline.

With the above solutions, the medium is made as follows: Place 300 cc. of the 10 per cent egg-white solution in a liter flask; 300 cc. of the 10 per cent egg-yolk solution in another flask; and 400 cc. of the meat infusion, to which is added 15 grams of powdered agar-agar, in a third flask. These are then sterilized in the autoclave at 15 pounds pressure for fifteen minutes. They are removed from the sterilizer and, while hot, 1 cc. of a 1 per cent alcoholic solution of gentian violet is added to the broth-agar. The contents of this flask are now poured into that containing the egg-white and then the egg-yolk is added. The whole is poured back and forth from this flask to another so as to insure thorough mixing and then it is tubed and slanted.

The tubes are left in their slanted position for about seventy-two hours at room temperature until the contents are well set. The cotton plugs are then trimmed and flamed and the tubes sealed with corks. This medium presents the same smooth inoculating surface as ordinary agar slants, contains as much moisture, is quickly made and is rendered absolutely sterile.

METHOD OF ISOLATING TUBERCLE BACILLI FROM SPUTUM

About 10 cc. of fresh sputum, which has been thoroughly washed in a running stream of sterile 0.85 per cent salt solution, is placed in a sufficiently large centrifuge tube containing a piece of blue litmus paper. An equal amount of 3 per cent sodium hydroxid is added and the whole well shaken. It is put in the incubator for about one hour or until the sputum is fairly well digested. The mixture is neutralized with normal hydrochloric acid, then centrifugalized, and after removing the supernatant fluid the sediment is planted on several tubes containing the herewith described medium by means of a large platinum loop or a capillary pipette. After from five to fourteen days incubation, a good growth appears which is free of any contamination.

This method has given us uniformly good results and the medium remains serviceable for at least one month.

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BACILLUS ABORTUS (BANG) AS AN ETIOLOGICAL FACTOR IN INFECTIOUS ABORTION IN SWINE

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So far as we are able to learn from the literature, the cause of infectious abortion in swine has never been determined. Lynch in his "Diseases of Swine," says, "Infectious abortion results from the infection of the genital passages by some specific germ, the true nature of which is as yet undetermined. The disease is not nearly so common as in mares and cows, and, while it may run through an entire herd, it is not likely to be spread from one farm to another except in unusual instances." He further states, "The infectious type of the disease is especially mild in its symptoms, and unless the animals are carefully watched the pigs may be slipped without any notice of the fact until several weeks later, when it is found that the sow is no longer pregnant." He also says, "The nearer to full term the sow is at the time of abortion, the less dangerous the occurrence and the more mild the symptoms. Signs of threatening abortion are loss of appetite, restlessness, making of the bed, shivering, trembling of the muscles, dulness, and in some cases very severe labor-pains."

While considerable work has been done with regard to infectious abortion in mares and cows in this laboratory, only three outbreaks of infectious abortion among sows, have come to our investigation. We have found that it is no uncommon occurrence for one or two sows in a herd to abort. Numerous cases of this kind have been studied by the writers, with no etiological results, which would lead to the opinion that such abortions were due to some accident.

In the large outbreak investigated by the writers early this year, some twenty sows aborted in rather quick succession. On visiting the place, it was found that one sow had aborted the night before, and two of the fetuses together with the attached afterbirth were brought to the laboratory for examination. The fetuses were fairly well developed, although not far enough along to have any hair. On one of the afterbirths there were noted numerous brownish, villus-like projections ranging in size from 1 to 3 mm. in diameter. They were so numerous in some places as to become confluent. On opening these, a dark serous fluid was noted. In our investigations upon the etiology of infectious abortion of animals, streak dilutions on agar or agar serum are always made and incubated under aerobic, Nowak, and anaerobic conditions. In this instance, streak dilutions were made on a series of agar plates, the agar being slightly alkaline to phenolphthalein, with material from the nodules on the afterbirth, from bits of the afterbirth from both pigs, and from the contents of the umbilicus, heart, liver, stomach and kidney of each fetus. From appearances, the kidneys of the pigs were very much enlarged and gorged with blood. As these pigs had lain on straw for a few hours, several of the plates showed on incubation in the air the growth of a considerable number of contaminating organisms, such as *B. coli*, *B. subtilis*, etc. This, of course is what would be expected on plating the afterbirth as the material had lain on straw for several hours before being secured by us. Cultures from the internal organs of the fetuses, however, were nearly sterile. The clear places on the plates were marked with India ink, after which they were subjected to the cultural method of Nowak.[1] The material was incubated at 37°C. for four days and removed from the jars. On casual examination of the petri dishes, we were led to believe that they contained nothing but the growth of bacteria usually encountered in plating material which had lain on the ground for some hours. Upon examining one of the plates carefully with a Coddington lens, however, there were noted in some of the clear spaces of the dishes a few very

[1] E. S. Good, Investigations of the Etiology of Infections Abortion of Cows and Mares, Bull. No. 165, Ky. Agri. Exper. Sta., 1912, p. 249.

small dew drop like colonies, which on being examined with the microscope resembled in every respect colonies of the *B. abortus* of Bang. Examined by the aid of the hand lens they were nearly water clear to direct light and of bluish tint to reflected light. They were round and raised, with exceptionally well defined borders. Most of these minute dew drop colonies were homogeneous, with the exception that a few of them had a few granules in the center, typical of many colonies produced by the *B. abortus*. Viewing the larger colonies under the microscope, it was seen that the centers had assumed a granular consistency, while the outer portion was homogeneous and transparent. The colonies on some of the plates were so large that they might have been taken for some other species, measuring $1\frac{3}{4}$ mm. in diameter. These conformed, however, to similar colonies of *B. abortus* derived from the tissues of the cow. To reflected light they had assumed an amber color, the centers having a whitish appearance. On examining stained preparations of these colonies, the morphology of the organism was identical, so far as we could determine, with *B. abortus*. On examining the plates carefully with a Coddington lens and microscope, colonies resembling those mentioned and measuring from a pinpoint to 1.5 mm. in diameter were seen on streaks made from the blood of the umbilicus, on the streaks made from the small nodule-like growths on one of the afterbirths, and from the afterbirth and internal organs of the fetuses. Eighty-four colonies in all were counted on streaks made from one of the small nodules. Some of these colonies were so small that they could not have been seen with the naked eye. There were one or two colonies on the streaks made from the livers. The plates streaked with the amniotic fluid were almost completely covered with contaminating bacteria, but, in the clear spaces five dew drop colonies were noted. The streaks of the contents of the stomachs of both pigs showed numerous small dew drop colonies. Streaks from the kidneys were negative. Stained preparations had been made from the different organs mentioned but were not examined until the cultures had developed. Upon examining these preparations, germs identical with the *Bacillus abortus* (Bang) were seen in large numbers in those made from the stomach contents of the pigs.

Typical colonies were streaked on agar slants, some of which were incubated by the Nowak method and others in the air. At the end of twenty-four hours, no visible growth was noted on the streak cultures incubated in the air. At the end of forty-eight hours, however, some little growth could be detected even with the naked eye, and at the end of seventy-two hours, quite a luxuriant growth of the organism was obtained, in appearance identical with streak cultures made with the *B. abortus*. On examining the tubes which had been incubated under the Nowak method, we found that they had made but slightly better growth than those incubated in the air. Streaks on agar at room temperature showed no growth in the time mentioned. We came to the conclusion that if this organism was the *B. abortus* and grew in the air after the first generation it was different from any we had ever isolated. The organism responded to the following tests in the following manner: It was found to be non-motile; gram negative; did not produce gas in either lactose or glucose; did not coagulate milk; grew readily in plain bouillon, showing a fair degree of cloudiness at the end of seventy-two hours; and did not liquefy gelatin. Serum-agar tubes heavily inoculated with this organism and quickly solidified in ice water and incubated in the air, developed a growth characteristic of the *B. abortus*, as noted by this laboratory, in that a narrow ring of growth appeared as a slight haze 3 mm. beneath the surface of the medium at the end of sixty hours, and eventually extended to the top of the medium. All the above tests conform to the biological and cultural characteristics of the Bang bacillus.

We were not, however, satisfied that the organism isolated was the *Bacillus abortus* on account of its growing in the air so readily, so we subjected the culture to the agglutination and complement fixation tests, using the serum from a rabbit made immune to the Bang bacillus, which agglutinated in high dilutions. It was found that this serum agglutinated our organism in a dilution of 1:1200, which was exactly the same dilution in which the serum agglutinated an antigen made of a well known strain of the *B. abortus*, which had been obtained from an aborting cow. Using as an antigen the organism isolated from the

sows and the immune serum mentioned, the complement was completely fixed with 0.02 cc. of serum. We were thus convinced that this organism was identical with the germ that produces abortion in the cow, the only difference being that it grew readily in the air after the first generation, while the cultures we have derived from the cow usually do not grow in the air until after being cultured for several generations by the Nowak method. An exception to this rule, was discovered by Dr. Frank M. Surface,[2] who accidently inoculated a cow with a culture of the Bang bacillus which had repeatedly been transplanted for some two years from agar to agar, or from agar to plain peptone broth, and then back to agar. He had obtained this particular strain while in Denmark. The cow injected, aborted and Surface isolated the organism, the first generation of which developed in the air. He was able to determine definitely, by using this organism as an antigen in the complement fixation test with an immune serum, that it was the Bang bacillus. Surface states that the growth obtained in a Novy jar (Nowak method) was in no respect better than that obtained in the free air.

We cannot state whether the organism isolated from the aborting sow would have grown directly from the tissues, as we cultured none of the material in that way for any length of time. Upon re-culturing the original material, which had been kept in the ice box, we found that the contaminating bacteria had become so numerous as to make streak dilutions impossible.

INOCULATION EXPERIMENTS WITH THE ORGANISM OBTAINED FROM THE ABORTING SOW

To test this organism further, a streak culture on an agar slant was washed with 5 cc. of physiological salt solution and 2 cc. of this material was diluted in 3 cc. of normal salt solution and injected intravenously into a pregnant sow, no. 1, on February 25, 1916. On March 13, seventeen days after the inoculation, this sow aborted five fetuses. The only symptom

[2] Surface, F. M., A Note on the Maintenance of Virulence by Bacillus Abortus Bang, Journal of Infectious Diseases, 1913, **12**, p. 359.

shown by this sow before aborting was that she did a great deal of rooting a day or two before slipping her pigs. As soon as she aborted she ceased rooting. The aborted fetuses, while quite well developed, were not haired over. Stained slides were made from the contents of the different organs. Streak dilutions were made of the heart, liver and stomach contents of each pig, as well as of the afterbirth, on 2 per cent agar poured in petri dishes and solidified. Some of the dishes were incubated in the air, while others were cultured according to Nowak. These fetuses were numbered 1 to 5. The organism with which this sow was injected was obtained from the heart, liver and stomach of pig no. 1; from the heart of pig no. 2; from the heart, liver and stomach of pig no. 3; from the heart and liver of pig no. 4; and from the heart and stomach of pig no. 5. Streak dilutions of these organs grown in the air showed no growth at the end of twenty-four hours. After forty-eight hours, however, the growth was distinctly visible to the naked eye, and at the end of seventy-two hours it was abundant. The streak dilutions grown under diminished oxygen (Nowak method) showed no more growth than that obtained in the air. Upon microscopical examination of the contents of the stomachs of pigs no. 1 to 5, it was seen that these organs harbored the germs in exceedingly large numbers.

On February 29, 1916, a pregnant sow (no. 2) was fed in shipstuff the growth of the organism, obtained from the aborting sow, on two large agar slants washed off with 40 cc. of sterile normal salt solution. This sow was kept in an inclosure separate from sow no. 1. On March 10, 1916, she received the contents of five small agar tubes in a similar feed. On March 17, the attendant informed us that this sow was going to abort because she was acting like the other sow, previous to aborting, in vigorously rooting the ground floor of her pen. On March 19, nineteen days after being fed the initial dose of the organism, the sow aborted. We obtained three of the pigs. She had eaten the afterbirth, and in all probability had also eaten some of the pigs, as she had bitten out a large piece from the side of one of the pigs secured. After a long series of dilutions, we were able to isolate the original organism from the stomach contents

of one of these pigs. The bacillus was present in this instance in very small numbers.

On March 27, 1916, the tails of these sows were carefully washed, shaved and disinfected, the ends cut off, and 25 cc. of blood taken. The blood serum of each of these sows caused complete agglutination of a known culture of *B. abortus* Bang in a dilution of 1:100, with 75 per cent agglutination in a dilution of 1:250, and the complement was completely fixed with 0.02 cc. of the serum. Serum from a normal hog tested at the same time did not agglutinate the agglutinating fluid in any dilution, nor did it fix the complement.

The slipping of the pigs produced no after effects upon the sows that we could notice. They will be kept under observation for some time.

Taking into consideration all the results mentioned in this paper, we may conclude that the *Bacillus abortus* (Bang) is an etiological factor in infectious abortion of sows. Whether or not it is the only etiological factor, will have to be determined by further investigations. This is the second time, so far as we know, that the *Bacillus abortus* has been associated naturally with aborting animals of a species other than the cow. The first was discovered by Dr. Surface[3] when he found the disease epizootic among guinea pigs which were being reared in an inclosure in which inoculation experiments were being carried on with the Bang bacillus. Some of the litter from the cages containing the inoculated pigs had gotten into the pens of breeding pigs and caused the spread of the disease.

SUMMARY

1. Epizootic infectious abortion occurs occasionally among sows, though not so frequently as among cows and mares.

2. Previous to the time of this investigation, no etiological factor connected with the disease in the sow had been discovered.

3. In this investigation the *B. abortus* of Bang, the organism

[3] F. M. Surface, Bovine Infectious Abortion Epizootic Among Guinea Pigs, Journal of Infectious Diseases, 1912, **11**, no. 3, p. 464.

causing the disease of infectious abortion in the cow, was isolated from the afterbirth of an aborting sow and from the contents of the umbilicus, heart, liver and stomach of two aborted fetuses.

4. The strain of *Bacillus abortus* isolated from the sow responded to all the biological and physiological tests of the strains isolated from the uterine exudate of aborting cows by this laboratory, with the exception that the original culture grew in the air after the first generation.

5. Pregnant sow no. 1, inoculated intravenously with 2 cc. of an agar slant culture of the bacillus secured from the aborting sow, washed off with 5 cc. of normal salt solution, aborted five fetuses seventeen days after the injection, and the organism was isolated from the afterbirth and internal organs of the fetuses. The bacillus in this instance grew directly from the tissues under strictly aerobic conditions. Pregnant sow no. 2, on being fed the organism derived from the aborting sow, aborted nineteen days afterwards. The sow ate the afterbirth and presumably some of the pigs. The organism was secured from the stomach contents of one of the fetuses obtained.

6. The blood serum of each of these sows, after aborting, completely agglutinated a strain of *Bacillus abortus* (Bang) derived from an aborting cow, in a dilution of 1:100. The complement was fixed in each case with 0.02 cc. of the serum. The serum of a normal hog did not agglutinate in any dilution, nor did it fix the complement.

THE RELATION OF PROTOZOA TO CERTAIN GROUPS OF SOIL BACTERIA[1]

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INTRODUCTION

The theory advanced by Russell and Hutchinson (1909; 1913) that protozoa are an important factor in limiting bacterial activity and consequent fertility in the soil has aroused no little interest. Their work has stimulated much investigation, some of the results of which seem to uphold their theory, while others do not substantiate it.

It was thought that results of interest might be obtained by further studies concerning the effect of the protozoa on certain biological processes of the soil—ammonification, nitrification and free nitrogen fixation.

For this study soil cultures of Miami silt loam soil from the Experiment Station Farm were used. The moisture content was maintained at as near one-half saturation as possible.

AMMONIFICATION

In this work 300 gram portions of soil were placed in each of 18 flasks. The flasks were of 500 cc. capacity, Erlenmeyer form, and rather narrow so that the soil was in approximately 2 inch layers. The proper amount of moisture was added, the flasks plugged, and sterilized at 15 pounds pressure for two hours. This was found sufficient to kill all the bacteria. Upon cooling, one half of the flasks were inoculated, each with 2 cc. of a suspen-

[1] Presented at Seventeenth Annual Meeting of the Society of American Bacteriologists, Urbana, Ill., December 29, 1915.

sion of normal soil known to contain protozoa. The remaining half were inoculated with the same amount of soil free from protozoa. The protozoa-free soil was obtained by sterilizing a portion of the Miami soil and inoculating it with as many kinds of bacteria as could be isolated by the plate method, using different kinds of media. The flasks were then incubated at room temperature (approximately 22° to 25°C) and the ammonia and nitrate content determined at the end of four, eight and twelve weeks. The ammonia was determined by distilling 100 grams of the soil with 10 grams of magnesium oxide and 250 cc. distilled water. The distillate was received into N/20 sulphuric acid and the excess acid remaining after the distillation was titrated with alkali of the same normality. The nitrate was determined by the phenolsulphonic acid method.

The results of this work are given in the following table:

TABLE 1

Ammonia and nitrate in soils with and without protozoa

TREATMENT	NITROGEN PER 100 GRAMS DRY SOIL					
	Ammonia			Nitrate		
	After 4 weeks	After 8 weeks	After 12 weeks	After 4 weeks	After 8 weeks	After 12 weeks
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
With protozoa.....	8.12	4.48	4.62	4.50	7.69	10.00
	7.70	4.69	4.90	4.50	7.69	10.00
	8.05	5.60	4.20	4.50	7.69	10.00
Without protozoa..	8.19	8.26	8.96	3.60	3.57	3.57
	8.10	8.19	8.96	3.60	3.57	3.57
	8.12	8.19	8.85	3.60	3.57	3.57

From the data of table 1 it will be noted that in the soil containing protozoa the ammonia decreased somewhat while in the soil free from protozoa it tended to increase to a slight extent. This may be explained by the fact that in the soil containing protozoa the nitrifying organisms were also present and functioning, whereas in the soil free from protozoa they were absent and thus the ammonia tended to accumulate. In the case of the nitrate formation, in the soil with protozoa the nitrate con-

tent naturally increased since the nitrifying organisms were present and the ammonia as it was formed was oxidized to nitrites and further to nitrates. Where the soil contained no protozoa, the nitrate content remained practically unchanged because of the absence of the nitrifying bacteria.

In order to show the correlation between ammonia formation and nitrate formation the following table giving the total ammonia and nitrate nitrogen was compiled.

TABLE 2

Total ammonia and nitrate nitrogen in soils with and without protozoa

TREATMENT	AMMONIA AND NITRATE NITROGEN PER 100 GRAMS DRY SOIL		
	After 4 weeks	After 8 weeks	After 12 weeks
	mgm.	*mgm.*	*mgm.*
With protozoa	12.62	12.17	14.62
	12.20	12.38	14.90
	12.55	13.29	14.20
Without protozoa	11.79	11.83	12.53
	11.70	11.76	12.53
	11.72	11.76	12.42

From the summary data in table 2 it is very evident that the presence or absence of protozoa has very little effect on the sum total of ammonia and nitrate nitrogen.

On account of the absence of the nitrifying bacteria in the protozoa-free soil it is hardly fair to draw very definite conclusions from these results. Therefore, other experiments were made, in which three sets of the same soil were used: (1) untreated, (2) heated to 90°C. for one hour and (3) heated to 90°C. for one hour and later reinoculated with 1 per cent of normal soil, thus introducing the nitrifying organisms and also the protozoa. These soils were incubated at room temperature and the ammonia and nitrate nitrogen determined every ten days for a period of thirty days.

The following results were obtained.

TABLE 3

Ammonia and nitrate in untreated, heated, and heated and reinoculated soils

TREATMENT	NITROGEN PER 100 GRAMS DRY SOIL					
	Ammonia			Nitrate		
	After 10 days	After 20 days	After 30 days	After 10 days	After 20 days	After 30 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated	2.10	1.82	3.50	4.63	5.55	9.80
Heated	8.96	9.80	11.75	2.50	2.51	3.03
Heated and reinoculated	8.96	10.08	6.44	2.96	6.66	8.33

As expected the ammonia content in the untreated soil remained quite constant while the nitrate increased. In the heated soil where the nitrifying bacteria and the protozoa were absent, the ammonia increased and the nitrate content remained practically the same. A part of the initial increase of ammonia in the heated and in the heated and reinoculated soils is undoubtedly due to the heating alone. The bacteria surviving this treatment cause some increase in ammonia as seen by the results obtained. But in the heated soil which was subsequently reinoculated with 1 per cent normal soil the ammonia decreased, due no doubt to its oxidation by the nitrifying bacteria, and the nitrate nitrogen increased. In the latter instance the protozoa were present, a factor which Russell and Hutchinson claim is detrimental to bacterial activity. The nitrifying bacteria were also present and active.

The following table compiled in the same manner as table 2

TABLE 4

Total ammonia and nitrate nitrogen in the untreated, heated, and heated and reinoculated soils

TREATMENT	AMMONIA AND NITRATE NITROGEN PER 100 GRAMS DRY SOIL		
	After 10 days	After 20 days	After 30 days
	mgm.	*mgm.*	*mgm.*
Untreated	6.73	7.37	13.30
Heated	11.46	12.31	14.79
Heated and reinoculated	11.92	16.74	14.77

shows the total ammonia and nitrate nitrogen present in the untreated, heated, and heated and reinoculated soils.

Here the introduction of the supposedly harmful factor, the protozoa, into the soil did not seem to have any depressing effect on the bacteria as far as their production of ammonia and subsequent oxidation of the same was concerned.

NITRIFICATION

Flask experiments were carried out somewhat similar to the ammonification tests, except that at the time of inoculation with soil containing protozoa and soil free from protozoa, the soil was also inoculated with cultures of *Nitrosomonas and Nitrobacter* free from ciliates and flagellates. The nitrifying organisms were obtained by inoculation and subsequent occasional transfer into media suitable to their growth and unfavorable to the growth of other organisms. After the inoculation of the sterilized soil as previously stated, ammonium sulphate was added in quantities equal to 20 mgm. of nitrogen as ammonia per 100 grams (dry weight) of the soil. The ammonia and nitrate content was determined at the end of fourteen and twenty-eight days.

The figures in tables 5 and 6 show the results of these determinations.

In the case of the soil with protozoa at fourteen days the average ammonia content was approximately 18.6 mgm. and at twenty-eight days, 16.0 mgm. Where the protozoa were ab-

TABLE 5

Rate of conversion of ammonia to nitrate in soil with and without protozoa

ANALYSES	TREATMENT	NITROGEN AS AMMONIA PER 100 GRAMS DRY SOIL	
		After 14 days	After 28 days
		mgm.	*mgm.*
1	With protozoa	20.58	15.82
2	With protozoa	19.02	15.96
3	With protozoa	16.10	16.24
1	Without protozoa	21.14	19.32
2	Without protozoa	23.24	19.18
3	Without protozoa	20.16	19.32

TABLE 6

Rate of nitrate formation in soil with and without protozoa

ANALYSES	TREATMENT	NITROGEN AS NITRATE PER 100 GRAMS DRY SOIL	
		After 14 days	After 28 days
		mgm.	*mgm.*
1	With protozoa	9.90	13.88
2	With protozoa	11.90	13.88
3	With protozoa	11.76	13.51
1	Without protozoa	7.52	7.81
2	Without protozoa	6.57	8.06
3	Without protozoa	8.20	8.06

sent the relative decrease in the amount of ammonia was about the same, 21.5 mgm. at fourteen days to 19.3 mgm. at twenty-eight days. There seemed to be practically no difference in the rate of conversion of ammonia into nitrate in the two soils.

Where the rate of nitrate formation was determined the increase in nitrate formation seemed to be slightly in favor of the soil which contained the protozoa.

From the small amount of work done on the effect of protozoa on nitrification in soil, it seems that their presence is at least not detrimental to the process as determined by these experiments.

FREE NITROGEN FIXATION

Some very interesting results were obtained in this part of the work. Here both soil and liquid cultures were employed. The liquid medium gave the protozoa an environment better adapted to their growth and activity than did the soil cultures.

Soil cultures. Four hundred grams of soil were weighed out and spread in approximately one inch layers on six flat porcelain plates. These were then carefully covered with parchment paper and tied and then sterilized at 15 pounds for one hour. Upon cooling each culture was inoculated with a suspension of *Azotobacter* in sterile distilled water. Then one-half were inoculated with a suspension of soil containing protozoa and the remaining half with the same amount of protozoa-free soil. Af-

ter incubating at room temperature for one week, the soils in the plates were treated with 1 per cent of mannite. The mannite was thoroughly mixed with the soil by means of a sterile spatula. The plates were then incubated for three weeks at 25°C. The moisture content was kept as near one-half saturation as possible by the addition of sterile distilled water. At the expiration of the incubation period, the soils were placed in a 30°C. incubator until air dry. They were then ground in a mortar and thoroughly mixed and finally sieved. Duplicate total nitrogen analyses were made according to the modified Gunning method.

The results of the analyses are given in the following table.

TABLE 7

Rate of fixation of free nitrogen in soil with and without protozoa

ANALYSES	TREATMENT	TOTAL NITROGEN PER 100 GRAMS OF DRY SOIL	
		After 21 days	Average
		mgm.	*mgm.*
1	With protozoa	148.40	
		145.60	
2	With protozoa	150.50	147.45
		144.20	
3	With protozoa	149.10	
		144.90	
1	Without protozoa	147.00	
		— —	
2	Without protozoa	144.20	145.02
		144.90	
3	Without protozoa	140.00	
		147.00	

From the data above it will be seen that there is a difference in total nitrogen in favor of the soil containing the protozoa. However, it is probably not marked enough to cause any difference in the fertility of the soil.

From these results it seems probable that the protozoa do not have any particularly harmful effect on the fixation of free nitrogen in the soil.

Liquid cultures. One hundred cubic centimeters of mannite

solution (Ashby's) were placed in each of ten liter Erlenmeyer flasks. To each of these flasks 10 grams of soil were added and the flasks and contents sterilized at 10 pounds for thirty minutes. After cooling each flask was inoculated with a suspension of *Azotobacter* in sterile distilled water. Finally, one half of the flasks were inoculated with 10 cc. of a suspension of 40 grams of normal soil in 400 cc. of sterile distilled water and the remaining half were similarly treated using protozoa-free soil. The flasks were incubated at 25°C. for three weeks. Previous to determining the total nitrogen the flasks were examined in order to ascertain whether or not protozoa were present. In those flasks inoculated with soil containing the protozoa they were present and in a very active state and in those inoculated with soil free from protozoa they were not found.

The results of the total nitrogen analyses are given below.

TABLE 8

Rate of fixation of free nitrogen in solution with and without protozoa

ANALYSES	TREATMENT	TOTAL NITROGEN PER 100 CC. OF SOLUTION	
		After 21 days	Average
		mgm.	*mgm.*
1	With protozoa	31.78	31.74
2	With protozoa	32.20	
3	With protozoa	31.36	
4	With protozoa	31.64	
1	Without protozoa	33.60	33.79
2	Without protozoa	33.60	
3	Without protozoa	34.61	
4	Without protozoa	— —	

The results of the total nitrogen determinations revealed a difference of 2.05 mgm. of nitrogen in favor of the cultures without the protozoa. Apparently the protozoa had a slight detrimental effect on nitrogen fixation in solution. The protozoa-free cultures contained 33.79 mgm. of nitrogen and the cultures with protozoa contained 31.74 mgm. of nitrogen.

Thus it appears that in liquid cultures where the protozoa are in an actively motile state they seem to exert a harmful

influence on the process of free nitrogen fixation. It is probable that the larger protozoa made use of the *Azotobacter* as food. In certain cases upon staining a small amount of the film from a liquid culture with Gram's iodine solution *Clostridium* cells could be very readily distinguished within the protozoan cell. The probable presence of *Azotobacter* cells within the protozoa cells was also observed but by no means as definitely as in the case of the *Clostridium*.

It may be concluded that the protozoa have a slight detrimental effect on free nitrogen fixation in solution because the individual determinations seem to check closely and to be quite outside the limit of experimental error considering the small amount of nitrogen in the cultures. In the case of the soil cultures such a slight difference is not so important because the total nitrogen content here is approximately five times that of the liquid cultures.

DISCUSSION

From the results of this study of the influence of the protozoa on ammonification, nitrification and free nitrogen fixation in soil it would seem that their effect can not be considered detrimental. This is in accord with the work of other investigators, even with that of Cunningham (1915) who claims that his results uphold the theory put forth by Russell and Hutchinson. In the work referred to he states that soil protozoa in solution exercise a decided limiting effect on the numbers of bacteria and that in an ammonifying solution they show their activity by causing a decrease in the amount of ammonia produced as compared with cultures free from protozoa. These results are in accord with those already presented in this paper, in regard to free nitrogen fixation in solution. The protozoa seemed to have a detrimental effect on this process but when experiments were carried out on free nitrogen fixation in soil, the protozoa did not appear to influence the amount of nitrogen fixed. Cunningham may well conclude that protozoa have a limiting effect on the number of bacteria in solution, for here an environment

is furnished for the protozoa which is never met with in soils under normal conditions.

The results of the work at the New Jersey Experiment Station are not in accord with Russell and Hutchinson's theory. Lipman et al. (1910) found that the protozoa do not play any important part in depressing the activity of the soil bacteria. This was shown by a series of experiments performed relative to a possible influence which the protozoa might have on the important soil process of ammonification.

Concordant results have been obtained by Sherman (1916) who worked with six species of protozoa, namely, the two ciliates *Colpoda cucullus* and *Balantiophorus elongatus*, which are not active in soil and four flagellates, which by test were shown to be active in soil. The ciliates had a very marked detrimental effect upon the number of bacteria in soil extract but had no effect upon them in soil. Three of the flagellates had no effect upon the number of bacteria either in soil extract or soil. The fourth flagellate had a very marked detrimental effect in soil extract but none in soil. These experiments were performed many times and always with the same results.

In an earlier work Sherman (1914) showed conclusively that some protozoa can increase in numbers in the soil under ordinary conditions but from the results of his later work it is probably doubtful if they have any appreciable effect on limiting the numbers of the soil bacteria.

Grieg-Smith (1912), drawing conclusions from his own work, thinks that the protozoa have but little effect on the bacteria of the soil. He tested the action of the soil phagocytes (the protozoa) in the same manner as Russell and Hutchinson did and from his experiments he concluded "that Russell's contention cannot be sustained; the protozoa have little or no action in limiting the number of soil bacteria."

Goodey (1911), working with the ciliates only, thinks that these protozoa do not exist in the soil in an active state, but that they are present in an encysted condition. He made a careful study of recently excysted *Colpoda cucullus* obtained from soil which had been added to a suitable medium but a few

hours before. He concluded that if these organisms had been in the soil in an active state they would have possessed food vacuoles, as these develop soon after the protozoan begins to ingest its food.

CONCLUSIONS

In conclusion it may be said that in the soil cultures the presence of protozoa under the conditions of the experiments did not have any noticeable effect, detrimental or otherwise, on the processes of ammonification, nitrification and free nitrogen fixation. In the case of the liquid cultures employed in the study of free nitrogen fixation the conditions were at an optimum for the development of the protozoa and under these circumstances they limited bacterial activity as evidenced by the harmful effect on the fixation of free nitrogen. Undoubtedly under these conditions the protozoa were active in destroying the *Azotobacter* cells. But in the soil cultures conditions were evidently not favorable for the activity of the protozoa as these organisms did not appear to exert any harmful influence on the three soil processes studied.

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A STUDY OF THE BOAS-OPPLER BACILLUS

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In 1895 Boas and Oppler (1895) published a paper in which they reported observations on a large bacillus occurring in the gastric juice of patients afflicted with carcinoma of the stomach. In the same year Schlesinger and Kaufmann (1895) found a similar bacillus in 19 cases out of 20 cases of gastric carcinoma examined. These findings were further confirmed by several investigators and the presence of large numbers of these bacilli in the stomach was taken to indicate carcinoma. Strauss (1895) reported finding similar organisms in normal gastric juice although in small numbers. Kuntze (1908) was the first to suggest that the Boas-Oppler bacillus was related to the lacto-bacilli. Rodella (1908) has also shown the similarity of the Boas-Oppler bacillus to the so-called acidophile or aciduric bacilli and the *B. bifidus* of Tissier. These suggestions were given further experimental support by the work of Heinemann and Hefferan (1908). The authors found in an extensive investigation of that group of bacteria, now commonly known as the *B. bulgaricus* group, that similar bacilli were present in two samples of normal gastric juice and in two cases of gastric carcinoma. These organisms are difficult to cultivate as they do not grow on ordinary laboratory media to an appreciable extent; but they grow well in milk or on media prepared from milk. The presence of glucose or some other carbohydrate favors growth. For a detailed description of the organism and its cultural characteristics the reader is referred to the publication of Heinemann and Hefferan (1908).

In 1914 Galt and Iles (1914) reported the finding of the same organism in three cases of gastric carcinoma. They thought that malignant cases of carcinoma could be distinguished from

benign ones by the presence of these bacilli. This conclusion was reached because in malignant cases the hydrochloric acid disappears, while lactic acid is frequently present.

Bacilli of the *B. bulgaricus* group are widely distributed in nature as shown by several authors. Heinemann and Hefferan have found them in the feces of man, horses and cows, in soil, in fodder for cattle (bran, silage, dry brewer's grains), in cornmeal, sauerkraut, olive juice, dill pickles, pepper mango, market milk and in human saliva. Hastings and Hammer (1909) reported the presence of these bacilli in milk, butter and cheese and recently Hunter and Bushnell (1916) ascribed the fermentation of silage to the activity of the *B. bulgaricus* group. That they are active in final stages of the ripening of Emmenthaler cheese has been shown by Eldredge and Rogers (1914) and they have been reported by Evans, Hastings and Hart (1914) in Cheddar cheese. Dotterrer and Breed (1915) during a tour of inspection of cheese factories in New York State have found that the pasteurized whey undergoes a practically pure lactic acid fermentation due to *B. bulgaricus* and that in unpasteurized whey the organisms are present in enormous numbers. The authors state also that the pasteurization temperature applied to these cases (66°–71°C.) was not sufficient to destroy the organism, although it destroyed most other bacteria present.

Since lacto-bacilli have been found in saliva and feces under normal conditions, it would be surprising if they did not exist normally in the stomach. Their presence in the digestive tract is the natural result of their frequency in foods, especially milk and milk products. Furthermore, this group of bacilli is able to resist a relatively high degree of acidity and survive where other bacteria are largely destroyed. Consequently the hydrochloric acid of the gastric fluid is not destructive to them, although it undoubtedly restrains their growth.

That lacto-bacilli actually exist in normal gastric juice has been shown by Strauss and Heinemann and Hefferan as stated before. If the hydrochloric acid restrains multiplication, it seems logical to assume that reduction in the amount of hydrochloric acid or its absence will permit growth and this assump-

tion may explain the finding of lacto-bacilli in large numbers in cases of gastric carcinoma.

However, if it be true that the presence of lacto-bacilli in large numbers in the stomach is due to a reduced quantity of hydrochloric acid, it may be assumed that they ought to be easily found in any pathological condition which reduces the normal acidity of the gastric juice. This reasoning led us to carry on a study of the problem the results of which are presented in this paper. We were aided by the kindness of Mr. A. G. Bower who furnished samples of gastric juice from a variety of sources.

The method of isolation was the same as the one employed by Heinemann and Hefferan. About half a cubic centimeter of gastric fluid was inoculated into acetic acid broth and incubated at 44°C. After twenty-four hours incubation several loopfuls were transferred to litmus milk and this was also incubated at 44°C. After a further twenty-four hours, transfers were made from the litmus milk tubes to other similar tubes and this proceeding was repeated every twenty-four hours until the characteristic reaction in litmus milk was observed. The coagulum should be smooth and compact with the appearance of little or no whey; the major part of the milk should be decolorized and a surface layer of intense red appears. Stains with methylene blue were prepared to ascertain the presence of long bacilli in pure culture. In order to study colony formation, plates were prepared in whey agar. Maltose broth was inoculated with five strains (P, S, F, H and the strain from infant feces) to test their reaction on this carbohydrate and finally 500 cc. of sterilized milk were inoculated with the same five strains. The evolution of acid was determined by titrating 5 cc. of the undiluted milk with N/20 NaOH and the acidity was calculated as lactic acid. The flask inoculated with strain H (from carcinoma) became contaminated and the results had to be excluded. The optical rotation of the lactic acid produced was determined after preparing the zinc salts in the usual manner.

Since lacto-bacilli are present in saliva it was suggested by Heinemann and Hefferan that they might be identical with

Leptothrix buccalis, the organism thought to be the cause of caries of teeth. We examined the decayed portion of four teeth after having carefully cleaned the teeth with sterile NaCl solution to avoid the chance of lacto-bacilli being present in the dried saliva on the outside of the teeth. After cleansing the teeth the inside decayed part was scraped out with a sterile knife and placed in acetic acid broth. Otherwise the same technic was employed as with the other material.

Material for examination was obtained from the following sources:

SAMPLE NUMBER	CONDITION	KIND OF MATERIAL	STAIN FROM MATERIAL
1. P.....	Gastric ulcer	Gastric juice	Many large granular bacilli
2. M.....	Gastric ulcer	Gastric juice	Many large granular bacilli
3. F.....	Gastritis	Gastric juice	Many large granular bacilli
4. S.....	Pernicious anemia	Gastric juice	Many large granular bacilli
5. H.....	Carcinoma	Gastric juice	Many large granular bacilli
6. P.....	No diagnosis	Gastric juice	Some large granular bacilli
7. J.....	Normal	Gastric juice	Few large granular bacilli
8. Bottle-fed infant.....		Feces	Many large granular bacilli
9. Breast-fed infant.....		Feces	Few large granular bacilli
10. Tooth.....	Abscess	Decayed part	No stain made
11. Tooth.....	Pyorrhea	Decayed part	No stain made
12. Tooth.....	Pyorrhea	Decayed part	No stain made
13. Tooth.....	Ulceration	Decayed part	No stain made

From all these cases typical lacto-bacilli were isolated. After four to six transfers in litmus milk the typical appearance of the milk was observed. Stains were prepared from all cultures and the bacilli appeared as large, rather slender or fairly thick organisms with blue granules. They were Gram positive. The colonies were all of the compact type. Sandberg (1904) first called attention to the two kinds of colonies formed by the Boas-Oppler bacillus. One of these has woolly edges, the other is solid. Similar observations have been reported by several authors.

The progressive amount of acid formed is shown in the following table:

SAMPLE	ORIGINAL MILK	AFTER DAYS								
		1	2	3	4	6	9	13	17	20
I. P.....	0.14	0.22	0.66	1.12	1.34	1.56	1.56	1.56	1.56	1.57
4. S.....	0.14	0.20	0.43	0.93	1.21	1.38	1.41	1.42	1.48	1.49
3. F.....	0.14	0.25	0.90	1.27	1.29	1.32	1.32	1.33	1.35	1.36
8. Infant feces	0.14	0.25	0.39	0.57	0.71	0.95	0.98	1.03	1.05	1.05

The amount of acid formed by the different strains is remarkably uniform and the rate of acid formation nearly the same in the first three samples. The strain from infant's feces is somewhat slower in acid formation than those from pathological conditions.

In maltose broth the strains P, S and F produced no change in reaction, while the strain from infant feces produced 4 per cent normal acid or 0.36 per cent lactic acid in five days at 37°C. and the same amount in two days at 44°C.

The strain from carcinoma lost its power to coagulate milk after seven transfers. We are unable to give an explanation of this phenomenon, unless it was due to enfeebling of the organism.

We found typical lacto-bacilli in the decayed contents of the four teeth. In one of the teeth (sample 10) long bacilli forming filaments and showing granular staining were found in large numbers. A few streptococci were also present. In one of the pyorrhea cases (sample 11) the bacilli were somewhat shorter than in the previous case. There were some that stained solidly, while others showed distinct granular staining. In the second pyorrhea case (sample 12) the long form was prevalent and filament formation was common. The fourth tooth (sample 13) was decayed and an ulceration was present at the root. The lacto-bacilli present were rather slender, the granular staining form being prevalent. Streptococci were also numerous. In the cultures obtained from two of the teeth (samples 10 and 13)

branching forms resembling the letter Y were observed. In all cases of material obtained from teeth the typical milk reaction resulted after three transfers in litmus milk. On account of the granular staining we assume that the lacto-bacilli from diseased teeth belong to the low-acid type.

This investigation confirms the results of previous work indicating that the Boas-Oppler bacillus is a member of the group of lacto-bacilli. Its cultural characteristics are in harmony with the descriptions of the group given by various authors and it appears established that members of the *B. bulgaricus* group are present in saliva, diseased teeth, gastric juice and the intestinal contents. The source can undoubtedly be looked for in certain foods, especially milk and milk products.

The finding of lacto-bacilli in large numbers in carious teeth is perhaps not conclusive evidence that they are actually the cause of decay. Experimental evidence to prove this would of course be difficult to obtain. However, since that kind of decay of teeth is usually ascribed to the presence of relatively large quantities of acid and since normal saliva is of an alkaline reaction the assumption is not difficult to arrive at that lacto-bacilli may be the cause. This is further supported by the fact that Heinemann and Hefferan found large numbers of lacto-bacilli in a sample of saliva of acid reaction.

White and Avery (1909) have attempted to separate the group of lacto-bacilli into two types as follows: Type A stains homogeneously with Löffler's methylene blue and Neisser's stain, produces 2.7 to 3.7 per cent lactic acid in milk and the lactic acid formed is of the inactive variety. Type B, stained with Löffler's methylene blue or Neisser's stain shows intensely stained granules; the bacilli of type B produce 1.2 to 1.6 per cent lactic acid in milk and the lactic acid formed is always levorotatory. According to this classification the strains examined by us belong to type B.

Rahe (1914) has classified aciduric bacteria according to their ability to clot milk and produce acid from maltose. He distinguished three varieties, namely: Variety A which clots milk, but has no action on maltose; variety B which clots milk and

ferments maltose; and variety C which ferments maltose, but does not clot milk. Strains F, P and S of our series belong to variety A of Rahe and the strain from infant feces belongs to variety B.

The presence of lacto-bacilli throughout the digestive tract has some bearing on the hypothesis of Metchnikof that life can be prolonged by establishing bulgarian bacilli permanently in the digestive tube. Rahe (1915) studied the problem of implanting *B. bulgaricus* in the alimentary tract and came to the conclusion that it cannot be adapted to the human lower intestine and that in monkeys the *B. bulgaricus* is capable only of an apparently limited survival in the upper intestine. The author further claims that *B. bulgaricus* can be readily distinguished from intestinal aciduric bacteria by its lack of ability to ferment maltose.

With these facts before us it seems clear that there is no support for the theory that lactic acid *in statu nascendi* is of value in suppressing intestinal putrefaction. The chief difference between the lacto-bacilli normally present in the alimentary tract and typical *B. bulgaricus* is the smaller amount of lactic acid produced by the former. However, the actual quantity produced is about 1.5 per cent, an amount which is greater than can be assumed actually to exist in the digestive tube. If we consider further that earlier findings of *B. bulgaricus* in feces, even after ingestion of bulgarian milk, were not entirely trustworthy on account of imperfect technic and that no attempt was made to distinguish between the high-acid and low-acid types, it becomes evident that the existence of an appreciable quantity of lactic acid in the digestive tract as a result of bacterial action is at least questionable. There can be no reasonable doubt about the actual formation of lactic acid by bacteria in the digestive tract, but the acid is promptly decomposed and utilized by the system.

The question naturally presents itself whether the types A and B of White and Avery are permanent or the result of environmental conditions and changeable. Type A is represented chiefly by typical *B. bulgaricus*. It forms about twice as much

lactic acid of the inactive modification as type B which produces only levo-rotatory acid. Granular staining is frequently a characteristic of enfeebled forms of bacteria and it is possible that type B is an enfeebled strain of type A that has lost the power to form dextro-rotatory lactic acid. If equal amounts of both modifications of active lactic acids are formed they unite to form inactive acid and it might be possible to convert type B into type A if suitable conditions of growth were offered. Type B is the one that is found active in cheese ripening and this fact suggests that type B grows at lower temperatures than type A whose optimum temperature is about 45°C. It is usually stated that type A does not grow below 30°C. It is possible therefore that type B is a modification of type A accustomed to lower temperature. We hope to study this problem at some future period.

SUMMARY AND CONCLUSIONS

1. Boas-Oppler bacilli occur in normal gastric juice in moderate numbers and in gastric juice containing either no hydrochloric acid or materially less than the normal amount in large numbers.

2. The presence of Boas-Oppler bacilli in large numbers in gastric juice is an indication of reduction of the normal hydrochloric acid content, whether this is due to gastric ulcer, gastritis, pernicious anemia or gastric carcinoma and possibly other pathological conditions.

3. The Boas-Oppler bacillus belongs to the group of lactobacilli which occur frequently in foods, chiefly milk and milk products. It gains access to the saliva, the stomach and intestinal contents with food.

4. The Boas-Oppler bacillus is the lactic acid producing organism that occurs in saliva and in the contents of the digestive tube.

5. The Boas-Oppler bacillus is common in feces of bottle-fed infants, but relatively scarce in the feces of breast-fed infants.

6. Four strains of Boas-Oppler bacilli studied by us produced in milk from 1.05 to 1.57 per cent acid, consisting chiefly of

lactic acid. No acid was produced in maltose broth by strains from pathological conditions.

7. The lactic acid produced by the strains of Boas-Oppler bacilli studied by us is of the levo-rotatory modification.

8. A strain isolated from the intestinal contents of a bottle-fed infant coagulated milk and produced 1.05 per cent (lactic) acid in twenty days. In maltose broth 0.36 per cent (lactic) acid was produced by this strain.

9. Lacto-bacilli were found in material from decayed teeth from which desiccated mucus had been removed. This fact suggests that *Streptothrix buccalis* is perhaps a lacto-bacillus of the low-acid forming type.

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A CONTRIBUTION TO THE BACTERIOLOGY OF SILAGE[1]

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The fermentation which ensilage undergoes during its curing process was looked upon a few decades ago as being entirely of microbic origin, and we find, in the older literature on the subject, frequent reference to the alcoholic, acetic acid and lactic acid ferments which were supposed to exist in the ensilage. The evidence upon which such statements were based was, as far as can be ascertained, merely the occurrence in silage of the products characteristic of the action of such organisms. At the present time, due chiefly to the work of Babcock and Russell (1900, 1901), opinion has swung in the opposite direction to such an extent that microörganisms are now generally considered of little if any significance in the normal fermentation of silage.

That most microbiologists in this country do not consider bacteria of significance in the formation of silage is indicated by a review of the recent textbooks on the subjects of general and agricultural bacteriology. Marshall (1911) and Jordan (1914) make no mention of silage, although other related fermented products are discussed. Russell and Hastings (1915) state that the fermentation is believed to be due to the respiration of the living plant cells. Lipman (1911) outlines the respiration theory of Babcock and Russell and states that silage may be made under conditions which exclude bacterial action.

[1] This paper, originally entitled "The Occurrence of Aciduric Bacilli in Corn Silage," was prepared for publication when a paper appeared by Hunter and Bushnell bearing a somewhat similar title. This article, slightly changed so as to contain reference to their work, is published only to confirm the observations of Hunter and Bushnell.

He further states that the question as to whether bacteria have any beneficial function can not be answered at the present time. Conn (1901) gives the old ideas concerning the supposed rôles of microörganisms, and then reviews the work of Babcock and Russell (1900, 1901). His views on the subject may be well exemplified by the following quotation:

> From all these facts it becomes clear that while this method of preparing food is due to a fermentation, it can not be attributed to the growth of microörganisms. It certainly involves other factors, and it is uncertain whether bacteria, or other microörganisms, have anything to do with the process as normally carried out.

Since the work of Babcock and Russell fifteen years ago, little has been published on the processes involved in the curing of ensilage. E. J. Russell's work (1908) was in substantial agreement with that of Babcock and Russell, though he thought it possible that bacteria play a minor part. Esten and Mason (1912) considered the process entirely bacteriological. Three chief fermentations were thought to take place: the lactic acid, alcoholic and acetic acid fermentation. The lactic acid fermentation was thought to be due to organisms similar to those concerned in the souring of milk. It was also believed by these workers that yeasts cause an alcoholic fermentation and that acetic acid bacteria then oxidize the alcohol so formed to acetic acid. Samarani (1913) concludes that the acetic acid fermentation in silage is due to the respiration of the plant cells, while the lactic acid fermentation is due to bacterial action. The organisms responsible for the latter process were identified by Samarani as a bacillus and a coccus which occurred in about equal proportions. The former he designated as the *B. acidilactici* of Hueppe, and the latter was considered identical with the common streptococcus of milk.

The rôle of acid producing bacteria of the Bulgarian type in such fermented products as sauerkraut, milk beverages, cheese, etc., is well known. That they should function in silage would appear probable, but until very recently no data were at hand which gave any evidence for such a belief. Although

suggestions that these organisms may be of importance in the ensiling of foods are found in the literature (Heinze, 1913), such suppositions have not been based upon established facts. The lactic acid bacteria mentioned by Esten (1910) as important in silage were inhibited by the presence of only 0.45 per cent of lactic acid when grown in corn juice. Gorini (1906) made a detailed study of the bacteria of silage and mentioned *Streptococcus lactis-acidi* and *B. lactis-acidi* as the most important of the acid-forming organisms. None of the organisms which he described had the property of forming a high degree of acidity. Löhnis (1907) in his classification of the lactic acid bacteria described a number of strains of the aciduric bacteria but made no mention of a group derived from silage. Stevenson (1911) studied the distribution of the high acid bacteria but did not report silage as one of the sources from which they were obtained. Heinemann and Hefferan (1909) noted silage as one of the substances from which they had isolated cultures of *B. bulgaricus*. The recent paper by Hunter and Bushnell (1916) however is the first report, so far as the writer is aware, of the constant occurrence and probable importance of organisms of the *B. bulgaricus* group in silage.

OBSERVATIONS

The notes recorded in this paper are those which have been made on ensilage, more or less incidentally, during the past year. It was first noted on April 26, 1915, that sterilized milk inoculated with silage developed a high acidity. After incubation for ten days at 37°C. the milk was found to contain an acidity of 2.3 per cent calculated as lactic acid. This observation indicated the presence of organisms belonging to the group of aciduric bacilli, and these bacteria were isolated from the milk culture by plating on ordinary lactose agar. Their occurrence has been repeatedly verified in samples of corn ensilage from four different silos taken at various stages during the feeding season.

That the high acid-producing organisms not only exist in

silage but that they occur in large numbers is shown by the fact that sterile milk usually develops a high acidity when inoculated with dilutions of silage representing only 1/1,000,000 of a gram of the moist material, or when inoculated with 1/1,000,000 of a cubic centimeter of the juice expressed therefrom. The occurrence of the high-acid organisms in such large numbers has been observed in silage over nine months old.

The aciduric bacilli may also be readily isolated by the direct plating of the silage on lactose agar on which this type can be grown. In fact these organisms constitute a majority of the bacteria found in ensilage during the latter part of the curing process. Unfortunately, it was not possible to make a study of the silage during the first two months when the most important bacterial processes undoubtedly take place. Plate counts made on lactose agar of the juice expressed from silage between three and six months of age have given counts ranging from a few thousand to over 1,000,000 bacteria per cubic centimeter. As is well known, most types of the aciduric bacilli do not grow well on agar plates, and it would seem very probable that the actual number of such organisms is considerably greater than is indicated by the plate count. That this supposition is correct is shown by the observation that these organisms may be present in numbers approximating 1,000,000 per cubic centermeter of juice,as revealed by the dilution method, when the plate count shows only 15,000.

Counts made on silage juice by the direct microscopic method of enumeration have shown the presence of from 1,500,000,000 to 4,800,000,000 bacterial cells per cubic centimeter, most of which were slender rods. Many of the organisms observed under the microscope were, in all probability, dead, since autolysis would undoubtedly be greatly retarded in such an acid medium. However, these observations indicate that immense bacterial activity had taken place.

The morphological and cultural characteristics of the acid producing bacilli which have been isolated agree with those reported by Hunter and Bushnell (1916). The rods were of variable size, but the growth characters of the cultures thus

far collected are very similar in so far as the cultural observations have been made. The colonies on agar appear exactly like those of the *B. lactis-acidi* group and, in the presence of a fermentable carbohydrate, they are surrounded by the characteristic haze. The development of colonies is not so rapid as with organisms of the *B. lactis-acidi* type, but on prolonged incubation they usually develop to a greater size. The readiness with which this group of bacteria grows on ordinary laboratory media differentiates it quite sharply from the typical *B. bulgaricus* of milk.

Not only do these organisms cause a high acid fermentation in milk but they have a similar action in corn juice in which they grow very rapidly. In the table given below are the data

TABLE I

Acidity produced by silage organisms in milk and in corn juice

CULTURE NUMBER	PER CENT ACID AS LACTIC ACID	
	Milk	Corn Juice
1	1.36	1.36
2	2.25	1.67
3	1.38	1.21
4	1.53	1.67
5	2.34	1.35
6	1.36	1.53
7	1.51	1.69
8	2.28	1.25
9	1.39	1.44
10	1.44	1.69
11	2.25	1.24
12	1.34	1.55
13	1.39	1.64
14	1.53	1.51

obtained with fourteen cultures grown in milk and in corn juice. The corn juice used was obtained from green plants at about the tasselling stage. The juice was expressed from the stalks by pressure, heated for a few minutes in the autoclave, filtered through filter paper, tubed and sterilized. The cultures were incubated twelve days at 37°C.

The observations reported in this paper would appear to indicate that acid producing bacteria might play a part in the fermentation of silage. How much of a factor they are in ordinary silage can not be answered from the meager data which have been collected. From the evidence presented by various workers, there can hardly be any question but that cell respiration is of vital importance in the fermentation of normal silage, but that this may be supplemented greatly by the action of bacteria certainly appears reasonable. It would seem that microörganisms might be responsible for the fermentation which takes place in silage made from shocked corn. The ensiling of shocked corn and corn stover, a practice which has been in vogue to a limited extent in some localities for years, in which we would expect the plant cells to be inactive, must be largely dependent, it would seem, upon the action of bacteria.

A laboratory test on this point was made by ensiling some corn stover with double the amount, by weight, of water in a glass jar. The stover used had been shredded and baled and was about fifteen months old. After one month at laboratory temperature the jar was opened and the ensilage examined. The material had a clean acid odor quite typical of ordinary silage, but on comparison of the two it was found to lack a certain richness in aroma so characteristic of silage put up in the usual way The juice expressed from the stover silage had an acidity of 1.35 per cent, calculated as lactic acid, and a bacterial count on lactose agar of 1,700,000,000 organisms per cc. of which 600,000,000 were of the high acid producing type. A direct microscopic examination of the juice revealed a count of 11,000,000,000 bacteria per cc.

The subject of the fermentation in stover silage is under further investigation at this station.

The constant occurrence of a group of organisms in silage with characteristics which differentiate its members from other related groups is of interest from a biological as well as from the practical viewpoint. The question naturally arises as to how they gain entrance to the ensilage, or what is their habitat in nature. Observations made in this connection indi-

cate an intimate relationship between the corn plant and this group of aciduric bacilli. Juice expressed from corn cut a few weeks prior to the time it was ready for the silo underwent an acid fermentation, when kept in the laboratory, with the production of a high degree of acidity and an aroma resembling that of good silage. From this were then isolated cultures of organisms similar to those obtained from silage. Sterile milk inoculated with bits of corn stover, either from the leaves or pith, always develops large amounts of acid and from it the typical organisms may be obtained. On a sample of shredded corn stover nearly a year and a half old the acid forming bacilli were found in numbers approximating 1,000 per gram as determined by dilutions in sterile milk.

SUMMARY

The data presented in this paper suggest the probable importance of a group of acid-tolerant, acid-producing bacilli in the curing of corn silage.

The organism concerned, while closely related to the *B. bulgaricus* group of milk and the *B. acidophilus* group of the intestines, appears to differ somewhat from the typical members of these groups, notably by its comparatively abundant growth on ordinary laboratory media.

The microscopic examination of silage juice demonstrates the presence of immense numbers of bacterial cells (always over one billion per cubic centimeter), most of which are bacilli which resemble morphologically the high acid producing bacilli described above.

The aciduric bacilli of silage are constantly found in quite large numbers on corn fodder, so that silage made from corn is always amply seeded with these organisms.

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BOOK REVIEW

Laboratory Manual in General Microbiology. WARD GILTNER. Pp. 418. John Wiley and Sons. 1916.

It is refreshing to have a laboratory guide for the study of microorganisms with a new title. This Manual is the result of the teaching experience of the author and co-workers at the Michigan Agricultural College and aims to be a guide for the teaching of general microbiology.

I suspect that many teachers experience the same difficulty the reviewer has felt in adapting another's laboratory directions to his own classroom work. The present Manual will be found extremely helpful and suggestive in the hands of the teacher but its wide acceptance as a class book seems doubtful, partly because of its length, and partly because of its lack of emphasis on any particular field or group.

The Manual is divided into three parts: Part I, General Morphological and Cultural Methods with 53 exercises; Part II, Physiology of Microörganisms with 33 exercises; Part III, Applied Microbiology, including 1 exercise on Air, 4 exercises on Water and Sewage, 10 exercises on Soil, 10 exercises on the Dairy, 1 exercise on Plant Microbiology, and 14 exercises on Animal Diseases and Immunity. The Appendix is of great value, including directions for the preparation of special media, stains, various tables and other data.

The illustrations are admirable and an excellent selection of pertinent references is given at the end of each exercise.

The book may be highly commended to teachers, who will cull from it many valuable suggestions, and when sufficiently extensive courses in general microbiology are offered it may well be adopted in the classroom.

C. M. HILLIARD.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

BACTERIOLOGY OF FOOD

Feeding Experiments with Bacterium Pullorum. The Toxicity of Infected Eggs. Leo F. Rettger, T. G. Hull and W. S. Sturges. Jour. Exp. Med., 1916, **23**, 475–489.

This investigation is divided into two phases: first, a study of the toxicity or disease-producing properties of *Bacterium pullorum* when administered orally; and, second, an investigation of the heat tolerance of this organism in infected eggs when prepared for the table by the usual processes of cooking. It was found that eggs which harbor *Bacterium pullorum* in the yolk in large numbers may produce abnormal conditions, when fed, not only in young chicks, but in adult fowls, young rabbits, guinea pigs and kittens. Many market eggs are infected with this organism and since such methods of cooking as soft boiling, coddling and frying on one side only do not render the yolks free from viable bacteria, the feeding of eggs thus prepared may be the cause of serious disturbances in persons who are particularly susceptible to such influences, and especially in infants. Inasmuch as the wide distribution of ovarian infection in the domestic fowl has come about only in the last few years, its possible danger to man is one of recent development.—B. W.

BACTERIOLOGY OF THE MOUTH

Observations upon the Endamoebae of the mouth; I. Endamoeba gingivalis (buccalis). C. F. Craig. Jour. Infect. Diseases, 1916, **18**, 220–237.

A general discussion of this protozoan, its historical significance, geographical distribution, morphological features, relation to *Endamoeba hystolytica*, and its pathogenic rôle. The author concludes that "it is more than doubtful that *Endamoeba gingivalis* is the cause of pyorrhea alveolaris and that it is yet too early to make positive statements regarding the relation of this parasite to disease."—P. B. H.

Pyorrhea Alveolaris and Some Modern Discoveries Regarding Its Causes and Treatment. F. E. Stewart. New York State Jour. Med., 1916, **16**, 71–79.

In a comprehensive discussion of pyorrhea alveolaris or Rigg's disease, the author calls attention to the prevalence of the condition, and its importance as an etiological factor in many systemic diseases. Microörganisms, either of virulent types or those ordinarily saprophytic in the mouth, may invade the living tissues and cause the infection.

Associated with pyorrhea alveolaris, various types of bacteria have been recognized for some years, and recently certain protozoa. A review of the more important contributions on the bacteriology and protozoology of these cases is given. Under treatment the use of ipecac and emetine as an amebicide against *Entameba buccalis* is discussed, likewise the use of ammonium bifluoride, as recommended by Head, as a bactericide. The use of bacterial vaccines (bacterins) is indicated, especially when the condition does not respond to local treatment, or when it becomes systemic. The technique of collecting the material for, and the preparation of, bacterial vaccines is outlined. In considering the use of autogenous and stock vaccines the author holds that in certain cases a polyvalent stock vaccine is sufficient. A discussion of the theoretical principles of immunity in relation to vaccine therapy is presented.—L. W. F.

BACTERIOLOGY OF SOILS

Variation in Nodule Formation. L. T. LEONARD. Jour. Amer. Soc. Agronomy, 1916, **8**, 116.

Varying degrees of susceptibility between varieties of the soy beans to inoculation regarding the production of nodules were investigated. Nineteen varieties of soy beans were taken and inoculated with 1 cc. of a broth culture of the soy bean organism. From the results obtained, the author draws the conclusion that a soy bean culture isolated from a single strain of soy beans will be successful in inoculating any of the varieties studied.—A. I.

Nitro-cultures and their Commercial Application. F. C. HARRISON. Trans. Roy. Socy. Can., 1915, Ser. III, **9**, Sec. IV, 219–223.

The writer refers to the paper presented to the Royal Society in 1906 by himself and Barlow on "The Nodule Organism of Leguminosae—its Isolation, Cultivation, Identification and Commercial Application." He reviews the commercial application of the methods then described, and notes that several firms in the United States have manufactured and distributed nitro-cultures according to these methods. The Ontario Agricultural College and the Macdonald College, Quebec, have sent out some 32,000 cultures with very favorable results, particularly with Alfalfa and Red Clover. Further experiments with media are reported, and the following revised medium which has been used successfully for the past two years is recommended.

Three solutions are prepared:

A. 75 grams of agar are dissolved in 3000 cc. of water, by placing in the autoclave at 10–15 pounds pressure.

B. 25 grams of hard wood ashes are boiled in 1000 cc. of water and filtered.

C. 0.5 gram of acid potassium phosphate, 0.5 gram of magnesium sulphate, 0.5 gram sodium chloride, 0.25 gram calcium sulphate, 6.25

grams of calcium carbonate are dissolved in the order named in 1000 cc. of hot water (about 80°C.).

The three solutions, A, B and C are mixed and 87.5 grams saccharose and 12.5 grams of mannite are added. The resulting 5 litres of medium are filled into ounce and a half wide mouth Blake bottles, plugged with cotton. These bottles when filled are sterilised in the autoclave at a pressure not exceeding 10 pounds. On removal, the bottles are sloped, and inoculated by means of a pipette. About 2 cc. of a suspension of the desired organism is run into each bottle. The bottles are kept in a sloped position, and incubated at 25°C. for about a week, when they are ready for distribution.—W. S.

BACTERIOLOGY OF WATER AND SEWAGE

Experiences in the Application of the Activated Sludge Process to Chicago Stockyard Sewage. ARTHUR LEDERER. Eng. Contrg., **45**, 388.

The temperature of the liquid affects the action in winter. The turbidity removal is an index of degree of treatment, and with the relative stability test aids in control.—L. P.

Winter Experience with the Activated Sludge Process at Milwaukee. W. R. COPELAND. Eng. Contrg., **45**, 386–387.

In winter with a sewage averaging 10.5°C. (minimum 4.5°C.) the oxidation was retarded. Two and one-fourth cubic feet air per gallon removed 90 per cent of bacteria and reduced the suspended matter to 15 parts per million. The sludge contained 5 per cent N as NH_3 and could be readily pressed. Analytical details are given.—L. P.

Grease and Fertilizer Base from Boston Sewage. R. S. WESTON. Jour. Am. Pub. Health Assn., 1916; **6**, 334–343; Eng. News, 1916, **75**, 913.

The use of SO_2 or H_2SO_4 on Boston sewage disinfects the liquid and precipitates most of the grease with the greater part of the suspended matter. Experiments showed a recovery of 1738 pounds dry sludge per million gallons containing 21.7 per cent grease. The estimates show an apparent profit of $6 per million gallons. Further investigation is recommended.—L. P.

The Sterilization of Chicago's Water Supply. Eng. Contrg., 1916, **45**, 18.

After 5 years trial, liquid Cl plants are to be installed on all intake lines. With less danger of tastes and odors, liquid Cl is cheaper, more flexible and capable of more precise control and reliability than other disinfectants.—L. P.

Maryland Experiences in the Disinfection of Water Supplies. R. B. MORSE. Eng. Contrg., **45**, 396–397.

From 0.2 to 3.0 parts per million of available Cl have been used in hypochlorite plants and 0.25 to 0.67 in liquid Cl plants. Fewer

complaints have been noted with liquid Cl. With intelligent operation the plants afford a cheap method of treatment for supplies not requiring filtration, and cause a marked decrease in typhoid.—L. P.

Copper Sulfate Treatment of St. Paul, Minnesota Water Supplies. N. L. Huff and G. O. House. J. Am. W. W. Assoc., 1916, **3**, 581–621.

One part per 12,000,000 parts of water was found to be adequate to eliminate *Spirogyra, Cyclotella* and most Cyanophyceae. One part per 10,000,000 remained effective for five weeks after which time treatment was repeated. Charts accompany the paper.—F. W. T.

The Water Supplies of Interstate Common Carriers on the Great Lakes. H. P. Letton. J. Am. W. W. Assoc., 1916, **3**, 364–384.

Letton discusses mechanical features of the subject together with various methods for treatment of such water. Bacteriological analyses were made according to the method promulgated by the Secretary of the Treasury (Public Health Reports, November 6, 1914, p. 2960). From these bacterial examinations Letton considers it an impossibility to obtain a drinking water for boats directly from the lakes that will at all times conform to the Treasury Department standard.—F. W. T.

The Use of Ammonia in the Chlorination of Water. Joseph Race. Can Engr., 1916, **30**, 345–346.

Ammonium hypochlorite proved to have a very rapid action, 20 to 30 times as rapid as $CaOCl_2$. The ammonium salt is not absorbed readily, preventing after-growths. The use of ammonia (aqua 16°B.) with $CaOCl_2$ may be economical when the $CaOCl_2$ costs over $2.08 per 100 pounds. Difficulties in application require quick mixing with large dilution after ammonia is added to bleach solution to avoid loss. L. P.

Some Aspects of Chlorination. Joseph Race. J. Am. W. W. Assoc., 1916, **3**, 439–449.

Among other subjects Race discusses the questions of aftergrowths and types of *B. coli* which survive chlorination. Much difference of opinion exists with regard to the question of aftergrowths. The aftergrowths, under usual working conditions, vary according to the dosage of chlorine employed. A small amount of chlorine exerts a selective action. With large amounts a flora of spore formers survives which is unlike the original flora of the water. A study to determine whether the *B. coli* found after chlorine treatment were more resistant, indicated that there was little difference in the resistance of various types to chlorine.—F. W. T.

A Study of Some Organisms Which Produce Black Fields in Aesculin—Bile-Salt-Media. F. C. Harrison and J. Vanderleck. With 4 plates. Trans. Roy. Socy. Can., 1915, Ser. III, **9**, Sec. IV, 207–217.

Six hundred pure cultures were isolated from aesculin bile-salt-agar, and lactose litmus agar plates made during analyses of 1000 milk sam-

ples from dealers or farmers in the Province of Quebec. These cultures were used to test the reliability of aesculin bile-salt-agar for the identification of the colon-aerogenes group. Of the 600 colonies many were selected because they seemed to be slightly atypical. There were only 10 exceptions; a percentage of 1.5 of all colonies tested; and a percentage of 0.03 of the colonies on the plates from which the 600 were isolated. Seventeen of the sub-cultures were subjected to the usual classification tests, and the results together with information as to the source of the milk are given in tabular form. Drawings showing the appearance on aesculin bile-salt-agar plates of surface and deep colonies respectively are included on plates I–IV.—W. S.

DISINFECTION

Further Investigation into the Precipitation of the Typhoid Bacillus by Means of Definite Hydrogen-ion Concentration. C. F. Kemper. Jour. Infect. Diseases, 1916, **18**, 209–215.

The aim of the present work was to ascertain whether there exists a specific hydrogen-ion concentration for the precipitation of all strains of *B. typhi*. None was found; the effective range of hydrogen-ion concentration was wide, most strains being precipitated in the presence of a 3.6 by 10^4 hydrogen-ion concentration derived from the dissociation of acetic acid in the presence of sodium acetate.—P. B. H.

Effect of Pasteurization on Mold Spores. Charles Thom. J. Agr. Res., 1916, **6**, 153–166.

The object of this investigation was to see whether mold spores could resist the heat applied in pasteurization. Several species of Aspergillus and Mucor, and a large number of species of Penicillium together with a few other kinds of molds were tested. It was found that nearly all the mold spores were killed either by the holder process (30 minutes at 63°C.) or by the flash process (30 seconds at 74° or 80°C). The flash process at 74°C. proved the most efficient, only occasional spores surviving.

The effect of dry heat was also investigated. Dried preparations of the spores were submitted to various degrees of temperature. The resistance to dry heat was found to be much greater than to moist heat, considerable numbers of the spores surviving unless temperatures of 120°C. (for 30 seconds) were used.—H. J. C.

The Physical Chemistry of Disinfection. J. F. Norton and Paul H. Hsu. Jour. Infect. Diseases, 1916, **18**, 180–194.

This contribution attempts to apply physico-chemical methods to the problem of the mechanism of disinfection; and in the first place to demonstrate the rôles which undissociated acids, hydrogen ions and anions play in the process. The first test was made with formic acid, using *B. typhi* as the test organism. The results seemed to justify the following conclusions: that acids act as disinfectants through the agency of the hydrogen ions, and that the disinfecting power is proportional

to the H ion concentration. The addition of a salt possessing a common anion diminishes the power through decrease in the H ion concentration and increase in the concentration of the undissociated acid molecules. Salts which do not appreciably affect the dissociation of an acid greatly increase the disinfecting properties. In acid disinfection acid anions are positive catalyzers and undissociated acid molecules are negative catalyzers.—P. B. H.

The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. I. The Problem of the Chemotherapy of Experimental Bacterial Infections. W. A. JACOBS. Jour. Exp. Med., 1916, **23**, 563–568.

Introductory to subsequent papers. A discussion of the factors to be considered in formulating a program for systematic studies in chemotherapy.

Ibidem. II. The Relation between Constitution and Bactericidal Action in the Substituted Benzylhexamethylenetetraminium Salts. W. A. JACOBS, M. HEIDELBERGER AND H. L. AMOSS. Jour. Exp. Med., 1916, **23**, 569–576.

By the addition of substituted benzyl halides to hexamethylenetetramine, a series of quarternary salts of this base was obtained. These salts represent a new group of organic bactericides. The results obtained in the tests with these substances upon *Bacillus typhi* have demonstrated the existence of direct relationships between chemical constitution and bactericidal action within the series. The bactericidal character is directly attributable to the presence of the hexamethylenetetramine nucleus. The degree of the bactericidal action, however, is determined by the position, character, and number of the groups substituted in the benzene nucleus. By the introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus of the parent benzyl hexamethylenetetraminium salt, the bactericidal power of this compound was notably enhanced. The substitution of these groups in the *ortho* position almost invariably resulted in substances which were more active than their *meta* or *para* isomers. The introduction of the methoxy group was without marked effect. Several substances in which two hexamethylenetetraminium side-chains occurred were found to be the most active of the substances of this series when tested against *Bacillus typhi*. Comparative tests with other bacterial types demonstrated that these compounds possessed a marked degree of specificity for *Bacillus typhi*.

Ibidem. III. The Relation between Constitution and Bactericidal Action in the Quarternary Salts Obtained from Halogenacetyl Compounds. W. A. JACOBS, M. HEIDELBERGER AND C. G. BULL. Jour. Exp. Med., 1916, **23**, 577–599.

The extension of the study of the quarternary salts of hexamethylenetetramine to those obtained by the addition of this base to the most

varied types of substances containing aliphatically bound halogen has demonstrated that the introduction of the hexamethylenetetramine nucleus in this manner results in the production of bactericidal substances or enhances the bactericidal action if already present.

In particular it was found possible by the use of the halogenacetyl group, XCH_2CO, as a connecting link, to furnish primary and secondary aliphatic and aromatic amines, alcohols, and hydrocarbons of the most varied character with the hexamethylenetetramine molecule and to study the relation between chemical constitution and bactericidal action in the series of substances so prepared. Because of the variety of chemical types studied, the results are too involved for a detailed summary here. Many of the substances were found to be very powerful bactericides, and in a number of instances derivatives of purely aliphatic nature were found to possess an unusual bactericidal power. *Bacillus typhi*, streptococci, meningococci, and gonococci were the microörganisms used for the tests, and striking instances of partial specificity were observed. This specificity was found to favor not one species alone, but instances were found in which each of the types of bacilli was shown to be especially susceptible to one or another of the particular types of compound employed. The source of this partial specificity is to be sought not in the hexamethylenetetramine nucleus itself but in the molecule to which it is attached. The action of some of the substances was tested in the presence of serum or protein and was found to be not at all or only slightly i ıhibited. In other cases marked inhibition occurred. The factors controlling the serum— or protein—compatibility of these substances are likewise to be sought in that portion of the molecule other than the hexamethylenetetramine.

B. W.

IMMUNOLOGY

Anti-Typhoid Inoculation. Eng. News, 1916, **75**, 530.

The Canadian Pacific Railway has used inoculation with success, reducing cases in two years to 3, as compared with 290 for two years among non-inoculated.—L. P.

Development of Immune Reactions in Serum Disease. W. T. Longcope and F. M. Rackemann. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 101–102.

Eleven patients, who had received horse serum for therapeutic purposes, were studied by two methods. Skin sensitiveness to horse serum was tested by intracutaneous injections of 0.02 cc. of horse serum, both undiluted and diluted ten or one hundred times with salt solution. Second, anaphylactic antibody was tested for by injecting the serum of the patient into guinea pigs and testing these for passive sensitization. The results show that anaphylactic antibodies for horse serum appear in the blood serum in maximum concentration towards the close of serum sickness and suggest that their presence determines recovery from this disease.—W. J. M.

Immunization with Sensitized Bacteria. HOMER F. SWIFT AND RALPH A. KINSELLA. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 103.

Two strains of green-forming streptococci were used. Rabbits were injected with sensitized vaccines and compared with other rabbits injected with unsensitized vaccines. In the latter group of animals there was strong formation of antibodies in from twelve to sixteen days. The animals injected with sensitized vaccines, on the other hand, showed only weak agglutinins or complement-fixing bodies and the serum was without protective value for mice.—W. J. M.

Complement Fixation in Acne Vulgaris. ALBERT STRICKLER, JOHN A. KOLMER AND JAY F. SCHAMBERG. Jour. Cutan. Dis., 1916, **34**, 166–178.

The authors call attention to the indefinite bacteriology of acne vulgaris and the probable etiological rôle of colon bacilli in this disease. They attempted a study of the relationship to the disease of the bacillus of acne, the cocci from acne lesions and of *B. communis* and *B. communior* from the feces of persons suffering with this disease, by means of complement fixation tests using the same antigens with the sera of normal persons and persons suffering with non-acneiform diseases and controlling the results in acne by testing the serum of their patients with polyvalent antigens prepared with cocci from furuncles and with colon bacilli from the feces of healthy persons.

Of 57 cases of acne vulgaris, 84.2 per cent reacted positively with an antigen of *B. acne*; 64 per cent reacted positively with the antigen of staphylococci from acne lesions and practically the same results were observed with the control antigen of staphylococci; 63.1 per cent reacted positively with the antigen of *B. coli* from the feces of acne patients and 32 per cent reacted positively with the antigen of *B. coli* from the feces of normal and healthy persons.

The sera of normal and syphilitic persons reacted uniformly negatively with all antigens; the sera of persons suffering with various skin diseases likewise reacted negatively in the majority of instances except those with acne rosacea and seborrholic dermatitis.

From these studies the authors conclude that *B. acne* may be an etiological factor in skin diseases other than acne vulgaris; that the cocci found in these lesions possess no peculiar serological characteristics such as would differentiate them from other staphylococci found in furunculosis and that *B. coli* appears to exert an etiological influence in some diseases of the skin and particularly acne vulgaris.—J. A. K.

The Variations in Reaction of the Blood of Different Species as Indicated by Hemolysis of the Red Blood Cells when Treated with Acids or Alkalies. J. G. CUMMING. Jour. Infect. Diseases, 1916, **18**, 151–179.

It had been shown in earlier work that the sign of the Wassermann reaction might be reversed by the influence of salts, acids and alkalies on the hemolytic system. The aim of the present work was to establish a chemical standardization for definite hemolytic time indices of

different animal species, as a preliminary to determining the percentage of ammonia, sodium hydroxide, hydrochloric acid and other inorganic and organic compounds and salts, necessary to cause complete hemolysis under certain arbitrary conditions. For a fifteen minute hemolytic system there was a marked difference in the requirements for NH_3, NaOH, and HCl, for some species but not for others. Various hemolytic time indices for the substances mentioned were worked out, and by this means it was found possible to identify blood cell suspensions of different species with considerable accuracy. The following conclusions were drawn: Alkaline hemolysis may be considered due to the hydroxyl group, while acid hemolysis is due to the H-ion. "The hemolysis of the red blood cell may be used an as indicator to ascertain the degree of acidity or alkalinity of certain solutions." "Alkaline hemolysis can be influenced by acids and acid hemolysis by alkalies. Both can be influenced by the neutral salt content of the suspension." It was shown that there was a distinct variation between the normal and the pathologic blood of the same species, since the time indices of the latter specimens were increased or decreased. It is suggested that this may be due to increased alkalinity or decreased acidity, or to variation in the natural salt content.

P. B. H.

INDUSTRIAL BACTERIOLOGY

The Removal of the Natural Impurities of Cotton Cloth by the Action of Bacteria. B. S. LEVENE, Journ. Ind. and Eng. Chem., 1916, **8**, 298.

Levene investigated the possibility of removing the nitrogenous and fatty impurities of cotton fiber by means of bacteria in place of the vigorous chemical treatment now employed. After preliminary experimentation the following organisms were found most suitable: *B. amylolyticus, B. fimi, B. bibulus, B. carotovorus, B. subtilis.*

These bacteria are capable of hydrolyzing starch, and decomposing cellulose or pectin or both.

Coarse cotton cloth was sterilized in nutrient broth inoculated with the above named bacteria and incubated at 37.5°C. Tests were made after one, two, and three months respectively. Slight changes were observed after one month, more marked effects after two months, and decided alterations after three months. Cloth washed and bleached was perfectly white and was not yellowed by steaming. Chemical tests showed complete removal of nitrogenous impurities, about 80 to 90 per cent of the ether soluble impurities, and from 2.5 to 40 per cent of the alcohol soluble substances. The effect on the last mentioned substances varied with the different types, *B. caratovorus* being the most effective. Two forms—*B. bibulus* and *B. fimi*—caused weakening of cloth; the others apparently had no such effect.

By using different combinations of organisms and different media the incubation period could be reduced to 24 to 72 hours.—I. J. K.

MEDICAL BACTERIOLOGY

The Pure Cultivation of Spirochaeta Icterohaemorrhagiae (Inada). T. Ito and H. Matsuzaki. Jour. Exp. Med., 1916, **23**, 557–562.

This organism, claimed to be the causative agent of Weil's disease, was obtained by the authors in a solid, a semi-solid, and a fluid medium, enriched with blood. The spirochaete thus isolated remains pathogenic for guinea pigs for many generations. The characteristics of three strains are described.—B. W.

A Milk-Borne Paratyphoid Outbreak in Ames, Iowa. Max Levine and Frederick Eberson. Jour. Infect. Diseases, 1916, **18**, 143–150.

The authors explain a slight epidemic on the grounds of a paratyphoid B. infection originating either from a carrier or from a convalescent. The paratyphoid cultures isolated, it is stated, produced gas in glucose broth.—P. B. H.

A Leptothrix Associated with Chronic Hemorrhagic Nephritis. G. R. Dick, G. F. Dick, and B. Rappaport. Jour. Infect. Diseases, 1916, **18**, 216–219.

The authors report a case of chronic nephritis characterized by recurring attacks of hematuria probably caused by a Leptothrix which was present in the urine. The causal relation was suggested by the constant occurrence of the organism, its virulence for animals and the improvement in the case which took place under vaccine treatment.

P. B. H.

Technique of Cultivating Human Tissues in Vitro. R. A. Lambert. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 100–101.

Chick plasma with the addition of an equal quantity of human serum furnishes a satisfactory medium, in which the fibrin network resists digestion. Human tissue may be preserved in viable condition for five to ten days, by immersion in salt solution in a cool place. The destruction of bacteria in infected tissues by means of chemical disinfectants is being investigated.—W. J. M.

The Cytology of the Exudate in the Early Stages of Experimental Pneumonia. Frank A. Evans. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 99–100.

Pneumonia was induced in rabbits by intrabronchial injection of pneumococci and streptococci and by the injection of egg yolk. The exudate in each instance contained many polynuclear cells but more often the predominant cells were mononuclear.—W. J. M.

Pneumotyphoid, with Report of a Case. F. Billings. Am. Jour. Med. Sc., 1916, **151**, 36–39.

Billings reports a case which "seems to present an example of typhoid fever presenting its primary manifestations in the lung in the form of

a frank, outspoken croupous pneumonia." Cultures from the blood, and from the sputum yielded *B. typhi*, but the organism was not found in the stools or the urine.—L. W. F.

The Mode of Infection in Pulmonary Distomiasis. KOAN NAKAGAWA. Jour. Infect. Diseases, 1916, **18**, 131–141, Pls. II–IV.

The author reports on the distribution, origin and means of infection in the case of the distomiasis observed in Formosa. It is shown that at least two species of crabs found in Formosa and Japan proper contain in the liver or gills large numbers of encysted larvae, and that the number of infested crabs in any district is roughly proportional to the number of cases of distoma infection. Dogs were successfully infected as a result of eating liver or lungs of infested crabs. In the final host it was shown that the encysted larvae, after entering the intestines, reach the abdominal cavity by perforating the intestinal wall near the jejunum. They then penetrate the diaphragm and pleura and finally pierce the lung parenchyma. Here they develop and lay eggs which are discharged with other degenerative tissue products through the trachea.—P. B. H.

The Reactions between Bacteria and Animal Tissues under Conditions of Artificial Cultivation. II. Bactericidal Action in Tissue Cultures. H. F. SMYTH. Jour. Exp. Med., 1916, **3**, 265–274.

In a previous paper the author described an original method for studying the action of tissue cultures *in vitro* when inoculated with living pathogenic bacteria. In the present experiments chick embryo tissues were grown in a mixture of equal parts of plasma and Ringer solution. It was found that chicken plasma exerts a marked bactericidal action on *B. typhi* and on *B. diphtheriae* but is less marked with *B. dysenteriae*, and slight, if present at all, with *B. coli*. Chick tissues, particularly splenic tissue, counteract this action. The migrating white cells from splenic cultures have a distinctly bactericidal influence on all organisms tested except *B. coli*.

Ibidem. III. The Action of Bacterial Vaccines on Tissue Cultures in Vitro. H. F. SMYTH. Jour. Exp. Med., 1916, **3**, 275–291.

In plasma tissue cultures *in vitro* with tissue containing lymphatic elements the changes characteristic of early tubercle formation may be seen when such cultures contain masses of tubercle bacilli.—B. W.

Experiments with Poliomyelitis in the Rabbit. M. J. ROSENAU AND L. C. HAVENS. Jour. Exp. Med., 1916, **23**, 461–474.

The poliomyelitic virus obtained from an experimental monkey was passed through eight generations in rabbits with no apparent change in virulence. It is filterable and is virulent only for young rabbits. Even in these only about 40 per cent succumb. Inoculations were made intracranially, intravenously, into the sheath of the sciatic nerve and by placing the virus upon the uninjured nasal mucosa.

The incubation period varied from two to forty-one days with an average of twelve days. The lesions produced while definite and consistent lack the distinctive features of the pathologic picture of poliomyelitis in man and the monkey. The symptoms differ in individual rabbits and show variations from those seen in the monkey and in man.—B. W.

The Protection of Pathogenic Microörganisms by Living Tissue Cells. P. Rous and F. S. Jones. Jour. Exp. Med., 1916, **23**, 601–612.

One series of experiments was carried out to determine whether phagocytes protect ingested bacteria against the bactericidal action of serum and of potassium cyanide. While the conditions of the tests are scarcely comparable with conditions in the body, yet they point to a protecting action on the part of the phagocyte. When erythrocytes and a hemolytic system were substituted for bacteria and bactericidal substances the results were sharp and conclusive. Suspensions of dog leukocytes were incubated with rat erythrocytes and dog serum was added for its opsonic action. After an hour's incubation anti-rat erythrocyte serum was added. It was then found that while all extra cellular rat erythrocytes were dissolved, the phagocyted red cells remained unaffected. From their experiments the authors conclude that living phagocytes are able to protect ingested organisms from the action of destructive substances in the surrounding fluid, and even from a strong homologous antiserum, and that this protection by phagocytes is largely, if not entirely, conditioned on their being alive. These findings should be taken into consideration in the study of diseases caused by infectious agents capable of living within tissue cells.—B. W.

Chemical versus Serum Treatment of Epidemic Meningitis. Simon Flexner and H. L. Amoss. Jour. Exp. Med., 1916, **23**, 683–701.

The authors studied the therapeutic effect of lysol and protargol in experimental meningococcus infections. Neither substance proved to have any curative action on the experimental infection in guinea pigs and protargol failed to influence favorably the infection following sub-arachnoid inoculation in monkeys. It was found that both lysol and protargol exert antileukotactic and antiphagocytic effects, and are also potent protoplasmic poisons, and the leukocytes with which they come in contact are injured and made to degenerate. The mixture of antiserum with lysol and with portargol reduces to a certain extent the antileukotactic and antiphagocytic effect of the chemicals; but this action is insufficient wholly to set aside the injurious effects which they produce. Any theoretical advantages they may possess are more than offset by the harmful effects which they cause, hence specific antiserum seems to provide the logical therapeutic agent with which to combat epidemic meningitis, since it is itself innocuous and promotes those processes essential to recovery from the disease.

B. W.

Further Experimentation in Animals with a Monilia Commonly Found in Sprue. B. K. Ashford. Am. Jour. Med. Sc., 1916, **151**, 520–528.

This report is a continuation of the author's studies in experimental moniliasis in animals by means of a new monilia found in sprue. He feels that sufficient evidence has been gained to justify Bahr's opinion that sprue is due to a monilia, but not *Monilia albicans*, since he has found in Porto Rico a distinct, undescribed species in nearly one hundred cases of true sprue, and in only a small percentage of carriers. This organism is designated as *Monilia X*, and is ordinarily of low virulence. The virulence on long cultivation is partially or completely lost, but may be recovered by passage through susceptible animals. When promptly injected, after recovery from patient with sprue, deaths generally result from mycotic septicaemia. Feeding tests with the freshly isolated organism from the patient ordinarily failed to kill animals, but when the virulence was raised by passage, it killed by this method of administration. Some of the animals died rapidly of a monilia septicaemia, others more slowly, probably from a toxin developed by a localization of the organisms in the intestinal tract; stomatitis has been observed, and also long continued severe diarrhoea following the feeding tests. Most of the tests were carried out on guinea pigs, although rabbits, monkeys, and the white rat were also used. The gross and microscopical findings of the autopsied animals are recorded. Noteworthy is the fact that the monilia, if attacking an internal organ, were seen as large colonies having the appearance of emboli. The intervening tissue spaces were generally free from the organism, thus radically differing from a bacterial septicaemia.

L. W. F.

Analysis of One Hundred and Thirty-Four Cases of Bacteriemia. M. Warren and W. W. Herrick. Am. Jour. Med. Sc., 1916, **151**, 556–577.

The author's report, as the title states, is an analysis of one hundred and thirty-four cases of bacteriemia observed during the past five years in the various services of a general hospital. An outline of the laboratory methods employed is given; also the bacteriological classification of the streptococcus group is considered. The cases are recorded under hospital case number, with age of the patient, the diagnosis, the maximum temperature, the leucocyte count and polynuclear percentage, the bacteriological blood findings, the treatment, and the result. Tables are given summarizing the authors' findings. Of especial interest to the bacteriologist is the table giving the General Summary, which follows:

General Summary

ORGANISM	NUMBER OF CASES	DIED	RECOVERED	IMPROVED	UNIMPROVED	DIED PER CENT
Str. hemolyticus	31	21	7	1	2	67.0
Str. viridans	40	25	10	2	3	64.0
Str. mucosus	1	1				100.0
Staph. aureus	39	22	14	3		56.0
Staph. albus	3	2	1			66.0
Pneumococcus	10	6	2		2	60.0
B. coli	6	4	2			66.0
B. influenzae	2	2				100.0
Anaerobic streptococci	3	2	1			66.0
B. mucosus	1	0	1			0
B. mallei	2	2				100.0
B. alkaligenes	1		1			0
Mixed infection	7	6	1			86.0
Str. hemolyticus and Staph. aureus	1	1				100.0
Str. hemolyticus and B. typhi	1	1				100.0
Str. viridans and Staph. aureus	1		1			0
Str. viridans and B. coli	1	1				100.0
Staph. aureus and B. coli	1	1				100.0
B. proteus and B. coli	1	1				100.0
B. influenzae and Staph. albus	1	1				100.0
All cases	134	83				61.1

In the table giving the results of treatment are found twenty-five cases treated with vaccines with a mortality of 81 per cent, and four cases treated with serum, with a mortality of 75 per cent, which according to the authors shows "the futility of present measures of specific therapy of generalized infections."—L. W. F.

Tonsillar Endamebiasis and Thyroid Disturbances. J. S. Evans, W. S. Middleton and A. J. Smith. Am. Jour. Med. Sc., 1916, **151**, 210–222.

The authors discuss the various theories of the causation of endemic goitre, in particular, those more recently advanced, based on the view that the disease is of an infectious nature. From data obtained from the physical examination of a large number (1328) of men (University of Wisconsin) it was found that 27.2 per cent had thyroid involvement. Of these, 22.8 per cent showed infective cryptic tonsillar lesions; this was increased to 90 per cent when those having nasal lesions were included. These observations suggest a connection between the nasal and throat affections, and the thyroid involvement. Since *Entameba gingivalis* (Gros) apparently plays a rôle in the etiology of pyorrhea,

certain cases of chronic tonsilitis and systemic complications, the writer's attention was directed toward the organism. In thirty-four cases showing typically diseased tonsils, 97 per cent showed entamebae in the crypts. Of this group, sixteen individuals were treated by means of emetin hydrochloride with a disappearance of the amebae from the crypts in thirteen cases (81 per cent). Emetin was administered to twenty-three individuals, sixteen of whom showed an appreciable reduction in the bulk of the thyroid. The group included seven dysthyroid cases; six were benefited in varying degrees. The improvement under the treatment with emetin led the writers to believe that an indirect relationship existed, and they concluded that "A symbiosis of entamebae with appropriate bacteria, leading to the elaboration and absorption into the thyroid of selective thyrotoxic poisons, is at least conceivable in explanation of such relation." But they do not consider this to be an exclusive explanation of all goitres. No entamebae were found in the thyroid gland.—L. W. F.